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331. THE EFFECT OF FEEDING SHARK-LIVER OIL TO COWS ON THE YIELD AND COMPOSITION, AND ON THE VITAMIN A AND CAROTENE CONTENT OF THE MILK

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Golding and his collaborators (1, 2, 3) were the first to show that the feeding of cod-liver oil to cows results in a depression of the fat content of milk. This effect was confirmed by Wagner & Wimberger (4) and, later, by several groups of workers in the United States (5, 6, 7, 8). The fatty-acid fraction of cod-liver oil is responsible for the effect (9), and hydrogenation of the oil prevents its specific fat-depressing action (8).

McCay & Maynard (7) found that shark-liver oil does not reduce the fat content of milk. As this oil is, moreover, much richer in vitamin A than cod-liver oil it offered better promise as a means of increasing the vitamin A content of milk. Experiments by Deuel and his associates (10, 11, 12, 13) in fact showed that the vitamin A potency of milk could be greatly increased by this means. These authors stated (10, 11) that, in addition to this increase, the administration of the oil was followed by an appreciable rise in the milk and fat yield.

These claims led us early in 1942 to an investigation of the effects of feeding shark-liver oil to dairy cows. In the meantime, and since, evidence has been accumulating in the United States confirming the findings of Deuel *et al.* regarding the marked rise in the vitamin A content of the milk but uniformly denying any beneficial effect of the oil on milk or fat yield (14, 15, 16, 17, 18, 19, 20).

EXPERIMENTAL

Materials and methods

Shark-liver oil is obtained from the liver of species of the family Squalidae, and contains 50–80% of non-saponifiable matter, compared with 0·8–1·0% in the liver oil of the cod (Hilditch (21)). The oil has little smell and, although it is very rich in vitamin A, contains but minimal amounts of vitamin D. The shark-liver oil used by us was obtained from the British Drug Houses Ltd., and our estimations showed that it contained 80,000 i.u. of vitamin A/g.

Ten mature Dairy Shorthorn cows were used as experimental animals. They were grouped in pairs, animals within each pair being as far as possible comparable with regard to stage of lactation, stage of pregnancy and initial milk yield. One cow in each pair was then allocated at random to treatment with shark-liver oil, the other cow acting as control. Details of the cows are given in Table 1.

The experiment lasted 7 weeks and was divided into four periods:

(1) A preliminary control period of 2 weeks when no oil was fed (23 February 1942 to 9 March 1942).

Table 1. *Details of the experimental cows*

Cow no.	Pair no.	Treatment	No. of calvings	Date of last calving	Weekly yield of milk (lb.)
F43	1	S.L.O.	3	23. ix. 41	135
P3	1	Control	4	20. ix. 41	118
P17	2	S.L.O.	2	13. ii. 42	219
L13	2	Control	3	12. xi. 41	227
P	3	S.L.O.	4	19. viii. 41	173
W7	3	Control	4	18. ix. 41	182
R5	4	S.L.O.	6	27. iv. 41	114
R6	4	Control	6	22. vi. 41	121
F50	5	S.L.O.	3	1. ii. 42	268
P19	5	Control	2	10. ii. 42	228

(2) A first experimental period of 2 weeks when the cows allocated to shark-liver oil treatment received 30 g. of the oil daily (9 March 1942 to 23 March 1942).

(3) A second experimental period of 1 week in which the oil ration was increased to 60 g. daily (23 March 1942 to 29 March 1942).

(4) A final control period of 2 weeks when oil feeding was stopped (29 March 1942 to 13 April 1942).

All cows were fed a standard basal ration throughout the experiment consisting of 8 lb. of meadow hay, 5 lb. of oat chaff, 43 lb. of mangels and 22 lb. of oat and vetch silage. In addition, a balanced concentrate mixture was fed at a rate determined by the milk yield of each cow. The ration of meadow hay was changed to an equal quantity of seeds hay 2 days before the commencement of the second experimental period. All food was weighed before feeding and any refusals were recorded. Throughout the experiment the cows had access to a bare pasture for a few hours each day for exercise.

The cows were milked into well-tinned pails, and all milk yields were recorded to the nearest $\frac{1}{4}$ lb. On 3 days of each week samples of morning and of evening milk were taken from each cow and the fat and the total solids determined. For the measurement of the vitamin content of the milk three pairs of cows were selected, as laboratory facilities were not sufficient to deal with samples from the whole ten. Morning milk was used, the sample being taken into a dark glass bottle. Ascorbic acid, riboflavin, aneurin, total carotenoids and vitamin A were determined on these samples. Reduced ascorbic acid was determined by a method already described (22). Dehydroascorbic acid was not measured as it was not likely to be present in appreciable quantities (23). Aneurin and riboflavin were measured fluorimetrically (Houston, Kon & Thompson (24)). Milk fat was extracted from the milk by the method of Gillam, Henry, Kon & White (25) and prepared for the measurement of vitamin A and carotenoids as described by Gillam, Henry & Kon (26). The measurements were made in a photoelectric spectrophotometer calibrated against pure β carotene and a vitamin A concentrate of known potency (Thompson (27)). Vitamin A was measured by the antimony trichloride reaction.

Results

The effect of shark-liver oil feeding on milk yield, milk fat, milk solids-not-fat, and on the condition of the cows

The shark-liver oil was weighed daily and mixed with the concentrate ration of each cow immediately before feeding. Three of the five cows showed no aversion to the oil, but two would not eat the mixture when first offered. One was induced to eat it but the other

had to be given the oil forcibly by mouth by syringe throughout the experiment. This cow, R5, refused all food on the first day of experiment with consequent temporary reduction of milk yield.

Table 2. *Mean daily milk yields in lb.*

	Preliminary period		1st experimental period		2nd experimental period	Final period	
Treatment	Week 1	Week 2	Week 1	Week 2	Week 3	Week 1	Week 2
Shark-liver oil	27·5	25·9	25·4	25·3	25·3	25·4	25·8
Control	25·2	24·9	25·0	24·9	24·7	25·2	25·9

Table 2 shows the mean yields of milk throughout the experiment. The feeding of shark-liver oil caused no change in yield at either the 30 or the 60 g. dose level. Statistical analysis of these results showed that the experiment was capable of measuring small differences in yield, for an increase in milk yield of 5% would have been significant when $P=0{\cdot}05$.

The fat percentages, the weight of fat produced per day, and the solids-not-fat content are shown in Table 3. There was a very slight net increase in the fat percentage and the fat yield of the cows fed shark-liver oil compared with the control cows, but this was not significant statistically. The solids-not-fat were not affected. Analysis of additional samples of milk taken during the last 2 days of the preliminary period showed that there was no initial change in the fat percentage associated with feeding of shark-liver oil.

Table 3. *Effect of the treatments on the mean percentages of fat and solids-not-fat of the milk and mean daily yields of fat in lb.*

		Preliminary period		1st experimental period		2nd experimental period	Final period	
Treatment		Week 2	2 days before feeding	Week 1	Week 2	Week 3	Week 1	Week 2
Shark-liver oil:	Fat %	3·87	4·13	3·98	3·98	3·77	3·83	3·84
	Fat yield	0·51	0·53	0·50	0·50	0·48	0·48	0·49
	S.N.F. %	8·89	8·92	8·95	8·83	8·80	8·83	8·80
Control:	Fat %	3·76	3·80	3·78	3·66	3·56	3·77	3·74
	Fat yield	0·47	0·47	0·47	0·46	0·43	0·48	0·48
	S.N.F. %	8·85	8·16	8·80	8·72	8·70	8·80	8·78

The cows were weighed at the end of the preliminary period and again at the end of the second experimental period. The control cows lost 2·6 lb. in 36 days and those fed shark-liver oil lost 1·4 lb. The difference in loss of weight is too small to warrant statistical analysis. The condition and health of the cows remained good throughout the experiment, and only one abnormality was noted. This affected cow P19 which had indigestion during the second experimental period. This was without effect on milk yield and the cause was not ascertained.

The effect of shark-liver oil feeding on the vitamin content of the milk

Water-soluble vitamins. Table 4 shows the concentration in whole milk of the water-soluble vitamins, ascorbic acid, riboflavin, and aneurin. There was no change in the concentration of these vitamins other than that due to a stage of lactation effect (24). It was noted, however, that during the estimation of aneurin by the thiochrome method the

fluorescence of the 'blanks' was higher in the milks of the shark-liver oil cows. This was probably due to fluorescence of vitamin A extracted into the isobutanol layer.

Table 4. *Effect of the treatments on the mean content of ascorbic acid, riboflavin and aneurin of the milk*

Treatment and period	Date of sampling	Ascorbic acid (mg./100 g.)	Riboflavin (μg./100 g.)	Aneurin (μg./100 g.) Free	Aneurin (μg./100 g.) Total
Shark-liver oil:					
Preliminary period	25. ii. 42	1·93	105	17·2	39·1
	4. iii. 42	1·92	106	16·6	35·9
1st experimental period	11. iii. 42	1·88	103	17·9	36·5
	17. iii. 42	1·85	120	17·8	33·8
2nd experimental period	24. iii. 42	—	—	—	—
	30. iii. 42	1·84	126	16·5	32·1
Final period	13. iv. 42	1·85	106	17·5	31·9
Control:					
Preliminary period	25. ii. 42	2·08	124	20·8	31·9
	4. iii. 42	2·00	121	20·9	39·7
1st experimental period	11. iii. 42	1·96	116	21·8	36·5
	17. iii. 42	2·02	122	22·6	36·2
2nd experimental period	24. iii. 42	—	—	—	—
	30. iii. 42	1·99	135	21·8	32·9
Final period	13. iv. 42	1·85	119	22·3	31·7

Rusoff, Skipper & Arnold (18) also found that the feeding of shark-liver oil was without effect on the level of ascorbic acid in the milk.

Vitamin A and carotenoids. An estimation of the carotenoids of the milk fat from bulked samples taken 15 hr. after the first dose of 30 g. of shark-liver oil showed that there was no change in either of the groups (Table 5). The carotenoid content of the fat of both

Table 5. *Effect of the treatments on the carotenoid and vitamin A content of the milk fat*

Treatment and period	Date of sampling	Carotenoids (μg./g. fat) F43	P17	F50	Mean	Vitamin A (i.u./g. fat) F43	P17	F50	Mean
Shark-liver oil:									
Preliminary period	25. ii. 42	3·30	3·10	3·70	3·36	19·6	18·2	17·2	18·3
	4. iii. 42	3·36	2·85	3·36	3·19	22·6	18·0	16·4	19·0
1st experimental period	11. iii. 42	3·42	3·30	3·50	3·41	148	110	132	130
	17. iii. 42	2·80	2·55	2·57	2·64	268	231	235	245
2nd experimental period	24. iii. 42	2·25	2·00	2·22	2·15	256	174	194	208
	30. iii. 42	1·54	1·54	1·86	1·67	390	316	360	355
Final period	13. iv. 42	4·60	2·00	4·32	3·64	36·6	32·0	31·6	33·6
Control:		P3	L13	P19	Mean	P3	L13	P19	Mean
Preliminary period	25. ii. 42	3·30	2·80	—	—	18·4	20·2	—	—
	4. iii. 42	3·70	2·80	2·20	2·90	20·8	21·0	24·0	21·9
1st experimental period	11. iii. 42	3·50	2·95	2·18	2·88	20·0	21·2	24·4	21·9
	17. iii. 42	3·12	2·60	2·00	2·57	22·0	20·8	25·1	22·6
2nd experimental period	24. iii. 42	3·50	3·75	3·10	3·45	27·4	26·8	33·2	25·8
	30. iii. 42	4·32	4·12	3·10	3·84	27·0	22·6	25·6	25·1
Final period	13. iv. 42	6·40	6·35	4·32	5·69	25·6	27·4	25·0	26·0

groups declined, however, during the first experimental period. When poor meadow hay was replaced by seeds hay of higher quality the carotenoid content of the milk fat of the control cows increased. On the other hand, the carotenoids in the milk fat of the cows fed

shark-liver oil declined at this time and increased considerably when oil feeding was stopped. The decrease of the carotenoids from the preliminary period to the last week of feeding was 50% for the cows fed shark-liver oil, while during the same period there was an increase for the controls of over 25%. It can therefore be concluded that the feeding of shark-liver oil depresses the carotenoid content of the milk fat.

Vitamin A was estimated 15 hr. after the first dose of shark-liver oil, when the concentration had already increased by 250%. The increases in the vitamin A content of the milk fat for individual cows are shown in Table 5. The results show that 40 hr. after feeding there had been a sixfold increase in the vitamin A concentration in the milk fat. A maximum appeared to be reached after 8 days on the 30 g. dose, averaging 245 i.u./g. of fat. The final test when the cows had been receiving 60 g. of shark-liver oil showed that the average concentration of vitamin A was 355 i.u./g. This high figure was confirmed by biological tests on rats carried out by Dr K. M. Henry, in which a value of 350 i.u./g. fat was obtained.

DISCUSSION

The results of the experiment show that the feeding of shark-liver oil was without effect on milk production, fat production or the percentage of solids-not-fat in the milk. This is contrary to the results of Deuel *et al.* (10, 11) but in agreement with the findings of other investigators (14, 15, 16, 17, 18, 19, 20). The oil had no deleterious effect on the health of the experimental cows or on their condition.

The phenomenal increase in the vitamin A content of the milk fat was greater than that reported by Deuel *et al.* (10, 11, 12, 13). In their experiments a dose of 1,400,000 i.u./day resulted in a maximum value of 170 i.u./g. of fat, and a dose of 4,200,000 i.u./day gave a maximum concentration of vitamin A in the milk fat of 280 i.u./g. In the present experiment 2,400,000 i.u./day resulted in a maximum value of 268 i.u./g. of fat and 4,800,000 i.u./day in a value of 390 i.u./g.

It is possible to calculate roughly the efficiency with which vitamin A was transferred to milk from the shark-liver oil consumed. The estimate can only be an approximate one, for it is based on the vitamin A concentration per g. fat measured on a single sample of milk, multiplied by the mean fat yields of the cows determined over a period of 3 days. With 30 g. of shark-liver oil the output was 5%; it fell to 3·6% when 60 g. were fed. Deuel *et al.* (13) found an efficiency of secretion of about 3%, while Jensen *et al.* (16) reported one of 2·2%. It is noteworthy that Rusoff *et al.* (18) were apparently unable to increase the vitamin A potency of the milk of their cows beyond about 45 i.u./g. fat with doses of shark-liver oil supplying from 795,000 to over 2,000,000 i.u. of vitamin A daily.

The depression of the carotenoid content observed by us in the milk fat of cows receiving shark-liver oil is in agreement with the findings of other workers in this field (12, 13, 16, 20). It had previously been observed by Martin, Cave, Whitnah, Beck & Atkeson (28) that a vitamin A concentrate of a potency of 500,000 i.u./g. exerted similar effects and there seems to be little doubt that this decrease in the carotene of milk is an effect of vitamin A *per se* (cf. (13)).

SUMMARY

1. An experiment with ten dairy cows has shown that the daily feeding of 30 or 60 g. of shark-liver oil containing 80,000 i.u. of vitamin A/g. is without effect on milk yield, fat yield, the fat and solids-not-fat percentage of the milk and on the health and well-being of the cow.

2. The concentration of aneurin, riboflavin and vitamin C in the milk was not affected by the feeding of shark-liver oil.

3. The total carotenoids of the milk fat decreased when shark-liver oil was fed. The vitamin A content of the fat increased 1100% when 30 g. were given and 1850% when 60 g. were fed. The highest individual value reached was 390 i.u./g. fat.

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332. THE COLLECTION OF QUARTER MILK SAMPLES BY MILKING MACHINE

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(With 1 Text-figure)

The collection of samples of milk from individual quarters has usually been done either by hand milking or by sampling by hand at the commencement of milking, before the application of the teat-cups for milking by machine. The former method is laborious, and may not be entirely accurate, as it depends a great deal on constant care throughout the milking to ensure that each quarter is milked into the correct container. If the quarters are milked separately by hand, the yield of milk from the individual quarters may be affected, and if the cow is not accustomed to being milked by hand, hand milking for quarter sampling may disturb her, and affect the yield, and so the composition of the milk. Quarter sampling by hand before the commencement of machine milking does not provide a representative sample of the yield of milk from the quarter, and it does not permit a measurement of quantity of milk produced by each quarter. In connexion with some work on the composition of milk from quarters showing a positive brom-thymol blue test (Hume (1), McDowall (2, 3)) and on the influence of such milk on the quality of cheese and butter made from it (McDowall (4)), the collection of full quarter samples of milk on a considerable scale was required. For this purpose a special milking-machine claw, a milking-machine bucket, and a strip bucket were constructed. These are illustrated in Fig. 1, I–IV.

(1) *The milking-machine claw.* The claw was of similar design to the normal milking-machine claw except that the milk connexions did not deliver to a single milk tube but were bent round between the straight tubes of the claw (Fig. 1, IV), so that they could be joined to four separate rubbers leading to the milking bucket. A small air-hole was bored in each of these bent tubes at the outside middle of the bend to allow ingress of air, and the normal air-hole in the claw was closed. The teat-cups were joined to the claw in the usual manner. The milking took place normally, the only difference being that the milk was drawn into the bucket through four rubbers instead of through only one. Four small glass-tubing inserts were connected into the four milk rubbers, about 6 in. from the claw, so that visual evidence could be obtained of completion of milking in each quarter.

(2) *The milking-machine bucket.* The bucket, made of tinned steel with a wired top, was cylindrical in shape (Fig. 1, I*a*), 17 in. high and 13½ in. in diameter. The outsides of both bottom and lid were slightly concave to give strength against atmospheric pressure when the bucket was under vacuum. Inside the bucket were four internal containers, 16 in. high, and in section the shape of a quadrant of a circle (see Fig. 1, I*b*). Each of these was provided with a small loop handle to facilitate withdrawal as required. The internal containers were all of uniform weight so that the weighing of the milk yield could be made in the container if this was desired. Each container had a capacity of 1¾ gal., which was found to be adequate.

The lid of the bucket (Fig. 1, II*a*, II*b*) was fitted to the wired top by means of a $\frac{3}{8}$ in. thick rubber gasket. It was provided with a central connexion flush with the inside and 4 in. high on the outside for connexion to the ordinary vacuum milk line of the machine, and with four additional connexions (milk dropper taps) symmetrically placed at two-thirds radius from the centre. These latter connexions protruded $1\frac{1}{2}$ in. through the bottom of the lid, so that when the lid was attached to the bucket with quadrant containers there was no possibility of the milk inlet to the bucket being placed above the dividing line of the quadrants, as the end of the inlet tube was $\frac{1}{2}$ in. below the top of these containers. The taps on the connexions facilitated application of the teat-cups to cows yielding milk from less than four quarters. An arrow head was painted on the lid and on the bucket at *X* (Fig. 1, II*b*). The quadrant containers were marked *A*, *B*, *C* and *D*, and

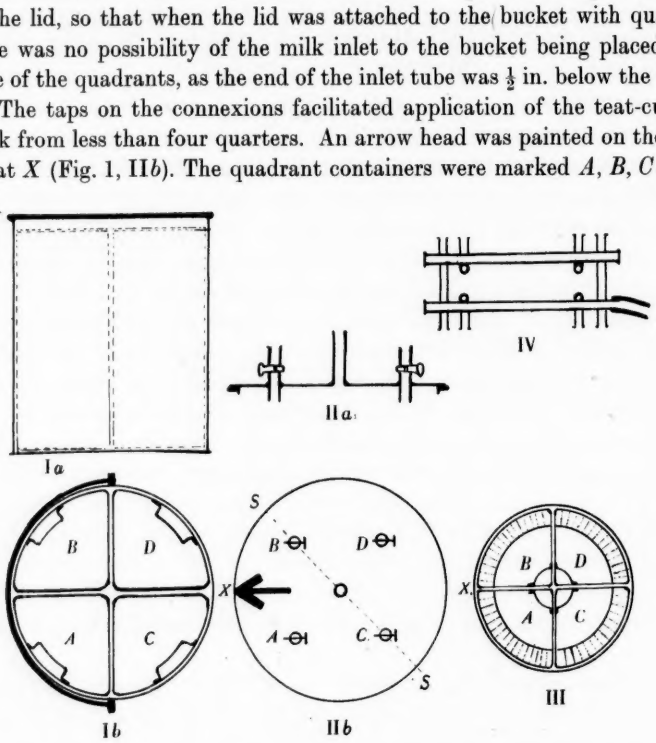

Fig. 1. Appliance for quarter sampling. I*a*. Elevation of quarter sampling bucket. I*b*. Plan of quarter sampling bucket with quadrant containers. II*a*. Elevation of section of lid through *SS* (see II*b*). II*b*. Plan of lid of bucket, with four tubelines, and taps, and central connexion. III. Strip bucket with tapering quadrant containers. IV. Special milking-machine claw with separate milk connexions for each teat.

were always placed in the bucket as shown with a diameter formed by the edges in line with the arrow at *X*, and the lid was applied so that the two arrow heads met. This assured that the quadrant containers were in the correct position in relation to the milk inlets.

(3) *The strip bucket.* The strip bucket was of normal bucket shape, and had four internal containers to fit (see Fig. 1, III). An arrow was painted on the inside of the bucket, and the containers were always placed so that a diameter formed by the edges was in line with the arrow. The containers were marked *A*, *B*, *C* and *D*, and were always placed in the same relative position to the arrow head at *X*.

(4) *Utilization of the appliance.* It was found that the use of the bucket in the milking shed gave very little trouble. It was necessary to ensure that the claw was correctly applied to the teats for delivery of milk to the four containers as labelled. In the work on

mastitis the four quarters were cited as *A*, *B*, *C* and *D* as follows: *A*, left front; *B*, right front; *C*, left hind; *D*, right hind. The simplest way to ensure that the claw was correctly applied was found to be the use of a standard position for the bucket relative to the direction in which the cow was facing. The bucket was always placed with the arrow on the lid pointed to the head of the bail, and care was taken that there was no twist in the long connecting tubes to the claw. The claw was applied to the teats with the straight tubes along instead of across the bail. With this system there was no need to alter the position of the bucket for right- and left-hand bails. It was found that there was little difficulty in using the claw as described instead of in the more usual position, transverse to the bail. Milking proceeded normally as for an ordinary bucket plant. The strip bucket was used in the same way with the arrow towards the head of the bail, so giving automatically an adjustment for right- and left-hand bails. On completion of stripping, the two buckets were placed side by side with the arrows pointing in the same direction, and the strippings were added to the corresponding quadrant container in the milking-machine bucket before weighing and sampling.

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333. THE COLIFORM FLORA OF MILK AND DAIRY PRODUCTS

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The coliform organisms have probably received more attention than most other groups of non-pathogenic bacteria owing to their importance as 'indicator' species in water analysis, wherein two distinct procedures have long been employed, viz. (*a*) a presumptive test to demonstrate the presence or absence of coliform species in general, performed by inoculation into bile salt-lactose broth (Britain) or lactose broth (America), and (*b*) confirmatory tests designed to isolate and differentiate faecal from non-faecal types.

Subsequently the 'coliform test' has been widely adopted in Britain for the examination of raw-milk supplies, and quantitative standards have been introduced for grading special designated raw milks. Similar tests are used extensively in pasteurized milk control, although no legal standard has been adopted. In order to facilitate examination of large numbers of samples, only the presumptive test is performed in dairy work. The validity of this procedure has been questioned by numerous workers, particularly in America, on the ground that interpretation of the test must be uncertain since faecal types are not differentiated. Conversely, it is argued that the presumptive test is sufficient since undesirable changes in milk or milk products may be produced equally by faecal and

non-faecal coliform organisms, so that the objects of the test differ from those involved in water analysis. Moreover, confirmatory tests would rarely be practicable in milk-control work involving large numbers of samples.

A considerable literature has accumulated dealing with various aspects of the coliform flora of raw milk, although comparatively few workers have proceeded beyond presumptive tests; an extensive review is given by Wilson (1). In contrast, very few investigations of dairy products appear to be recorded. Some attention has been devoted to defective products, but few systematic investigations of the normal incidence of coliform types have been recorded. The control of coliform organisms in dairy products is undoubtedly of considerable importance to many bacteriologists and technicians, whose examinations almost invariably include a 'coliform test'. Further information concerning the significance and interpretation of such coliform tests would appear to be of some importance.

Various difficulties involved in the performance and interpretation of the coliform test may be summarized here:

(1) False presumptive positive tests yielded by spore-forming anaerobic bacilli have long been observed in water and soil analysis, particularly when (as in America) lactose broth is used. Hall & Ellefson (2), and Salle (3) have proposed the addition of brilliant green, gentian violet, or crystal violet to the test medium as inhibitors, but Stark & England (4) found that this procedure suppressed some faecal coliform types. Poe (5) found that addition of bile salt to the medium did not eliminate anaerobes and sometimes encouraged their growth. However, Wilson (1), in an extensive, critical examination of the coliform test, states that bile salt-lactose broth yielded few false positives from water or milk, and this is probably the common experience in Britain. Some dairy products are subjected to heat treatment during manufacture, and the occurrence of significant numbers of false positive tests seems theoretically possible, particularly since spore-forming organisms, e.g. *Cl. welchii*, are not uncommon in milk. Plant-control measures may be dependent upon correct interpretation of tests, but this possible error seems largely to be ignored. False positive tests yielded by yeasts have also been recorded, but appear to be infrequent.

(2) Numerous diverse coliform types are known, and it is doubtful whether all are of faecal origin. Simplified classification into a few well-defined general groups has now somewhat reduced confusion. Thus Parr (6) suggests an American classification of five groups only, viz. *coli*, intermediate, *aerogenes*, *cloacae* and *klebsiella*. Malcolm (7, 8), in Britain, classified strains isolated from milk into *coli* (one type), *aerogenes* (one type), *cloacae* (one type) and intermediate (11 types). The British Ministry of Health (9) suggested that seven types are adequate for water-analysis requirements, viz. *coli* (two types), *aerogenes* (two types), intermediate (two types) and *cloacae*. Wilson (1) investigated the suitability of the coliform test for grading milk and proposed the addition of eight 'irregular' types to the seven types employed in water analysis.

All workers agree that the *coli* type constitutes the normal coliform flora of human and bovine faeces, although Wilson (1) found the type I strain of undoubted faecal origin, whilst the type II strain was infrequent in faeces but common in hay and straw.

The *aerogenes* and *cloacae* types appear to be common in soil and vegetation, but infrequent in faeces if ordinary methods of isolation are used; thus Malcolm (7) found only 3·5% *aerogenes-cloacae* and Wilson (1) only 2% *aerogenes* strains amongst organisms isolated from faeces. However, Tonney & Noble (10) had shown that these strains could be

isolated readily from dung if special enrichment methods were used, and this finding was confirmed by Malcolm (7), who obtained citrate-positive types from 95% of samples by special enrichment and suggested that such types normally occur in small numbers in bovine faeces.

Intermediate types have been widely studied, and Werkman & Gillen (11) considered that the citrate-positive types constitute a distinct group for which the generic name *Citrobacter* was proposed. Tittsler & Sandholzer (12), working with a small number of cultures, found no single distinguishing characteristic and suggested allocation of intermediates to the *Aerobacter* group. The original habitat of intermediates remains uncertain. Wilson (1) found them the commonest coliform type in soil, but absent from cow dung. Parr (13) studied a large number of intermediate cultures and regularly found members of the group in faeces, but could not determine whether they were essentially of faecal origin.

Parr (6) also suggested that changes from typical *coli* to citrate-positive intermediates, and vice versa, may occur in soil. He further pointed out that *aerogenes* and intermediate types predominate in urine, and increase at the expense of *coli* types when faecal samples are stored. The present investigation has shown that intermediates occur in dairy products.

Finally, a recent ecological survey by Griffin & Stuart (14) of the distribution of coliform organisms, covering a very large number of strains, has emphasized the importance of environment and geographical factors. Moreover, statistical evidence suggested that *Aerobacter* and intermediate types constitute the normal coliform flora of soil and the *Escherichia* (*coli*) types that of faeces; occurrence outside these sources was believed to be adventitious. Parr (6) states that in general coliform bacteria are ubiquitous, and certain types best adapted to any given environment will predominate there, but all coliforms must be thought of as possibly faecal in origin.

No wide ecological survey of coliform strains in manufactured dairy products appears to have been published in Britain. An investigation of this type was described by Yale (15) in America, possibly under different environmental conditions; moreover, the number of samples examined was small and the methods of differentiation adopted would probably be criticized by many workers.

(3) The quantitative estimation of coliform organisms in milk is subject to serious sources of error which do not arise in water analysis. Wilson (1) has pointed out that the dilution method commonly used may involve large sampling errors, whilst there are possibilities of overgrowth by individual species both during storage of the sample before testing and in the test medium itself, in addition to the danger of 'diluting out' individual species. For all these reasons a completely false picture of the original coliform flora may be obtained. In practice the theoretically sound method of direct plating is unsatisfactory, since no reliable and selective solid medium has been devised. Wilson (1) suggested two modified dilution methods, but as yet these have not been generally adopted. The principle used in the examination of dairy products resembles that commonly employed for raw milk, i.e. the normal dilution method without differentiation of presumptive positives. In spite of the errors involved, some further knowledge of the coliform types responsible for presumptive positive reactions appears desirable.

(4) A difficulty peculiar to dairy work arises from the occurrence of 'low-temperature' strains (usually *aerogenes* or *cloacae* types) which produce gas from lactose at 25–30° C. but not at 37° C. These strains were reported almost simultaneously by Grimes (16) from butter and Hiscox (17) from gassy cheese. Allen & Harrison (18) also isolated them from

grass, silage and raw milk, and suggested that bile salt may be inhibitory at 37° C. (Wilson (1) showed that bile salt is slightly inhibitory even for normal 37° C. types.) Later Oxley (19) showed that such strains occur commonly in raw milk.

(5) The existence of 'heat-resistant' coliforms, though of no significance as regards actual testing methods, may be important in dairy-plant control, and recently this problem has attracted some attention. The heat resistance of coliform organisms in milk had been studied by, among others, Ayers & Johnson (20), Tanner & Windsor (21), Wilson (1), and Vernon & Walker (22); in ropy milk by Hammer & Hussong (23); and in ice cream by Nelson *et al.* (24) and Paley & Isaacs (25). The relationships between growth phases and heat resistance in milk have been investigated by Elliker & Frazier (26). All workers agree that heat-resistant coliforms found in milk or ice cream are mainly *Escherichia* (*coli*) types.

The incidence of heat-resistant coliforms may be of practical importance in dairy manufacture, but has not as yet been widely studied.

(6) The present investigation has revealed irregular incidence of coliform strains in dairy products. A particular strain may occur in the product from a manufacturing plant for several consecutive days, followed by complete absence for a long period; this is apparently due to sporadic plant infection. If, therefore, a limited range of samples is examined, false estimates of the frequency of particular strains may be obtained.

Objects of the investigation

The present investigation has been concerned with the incidence and sources of coliform types in dairy products produced by large-scale manufacturing methods, bearing in mind the possible significance of difficulties outlined above and their relation to interpretation of results and plant control. Wherever possible, the investigation was not confined to finished products but was extended to cover various stages of manufacture. The study has been spread over 11 years in order to include a wide range of samples and to avoid difficulties arising from seasonal variation or irregular incidence of strains in manufacturing plants. Attention was devoted mainly to normal products of known history manufactured by a group of factories in south-west England, and it was hoped that useful comparisons would be obtained between different products manufactured from a common milk supply.

Experimental methods

(1) *Presumptive test*

The normal dilution method in bile salt-lactose (MacConkey) broth was used because it was the standard method in Britain and information concerning it appeared desirable. Moreover, some results would be comparable with the few recorded observations of other workers. (The modifications proposed by Wilson (1) had not been published when this work was commenced.) It should be noted that studies carried out in America have been based upon a presumptive test employing lactose broth without bile salt.

The quantities inoculated into MacConkey broth were varied as required to cover the expected quantitative range of coliform organisms present in each particular product; in some cases a wide range of dilutions was necessary, whilst in others heavy inocula (e.g. 10–50 ml.) were required to demonstrate the presence of coliforms. Incubation was carried out for 3 days at 37° C.

(2) *Isolation of cultures*

The use of MacConkey broth as the preliminary enrichment medium is an essential feature of the British standard method, but beyond this stage there seemed no strict reason for the use of standard MacConkey agar for isolation of cultures. Trials were made of other solid media which have been proposed to facilitate differentiation, although some of these have been designed to restrict growth to one type only, e.g. *coli* or *aerogenes*, which was not desired in this work. None proved entirely satisfactory in the author's hands, but Levine's eosine methylene blue agar (27) was finally chosen because it seemed to yield more recognizable colony types than did standard MacConkey agar, whilst all the main coliform types developed readily upon it. It is easily prepared and has been adopted widely in America by both dairy and water bacteriologists.

Eosine methylene blue agar plates were streaked from positive MacConkey broth tubes; in all cases the highest positive dilution was streaked and in many cases the lowest dilution also. The plates were incubated at 37° C. and examined after 24 and 48 hr.; the longer incubation was usually preferable and occasionally essential. Each type of colony was removed for further examination and purification, whether its macroscopic appearance suggested a coliform type or not. This stage was extremely laborious owing to the common occurrence of mixed colonies, particularly from raw milk; the isolation of pure cultures on E.M.B. agar often proved a tedious and lengthy procedure, involving many replatings. In some cases cultivation in peptone water before each replating proved useful, whilst in others pure cultures were separated more readily on standard milk agar than on E.M.B. agar. Ruchhoft *et al.* (28) experienced similar difficulty with mixed colonies in water analysis.

Some E.M.B. agar plates showed either no colonies, or cocci only, after 48 hr. incubation at 37° C. During the early stages of this work it was accidentally discovered that some such plates developed colonies after standing a few days at room temperature; these proved to be coliform organisms, usually *aerogenes-cloacae* types, presumably similar to those reported by Grimes (16) and Hiscox (17). Thereafter, when negative plates were obtained at 37° C., replatings were made at 22° C. Using this procedure, low-temperature coliforms able to yield a positive reaction in MacConkey broth at 37° C. but capable of only feeble or no development on E.M.B. agar at 37° C., were isolated from both raw milk and manufactured dairy products. In thirty-one instances only, yeasts able to produce gas in MacConkey broth were isolated.

Other presumptive positive tubes yielded no coliform colonies in spite of several replatings. Search was then made for anaerobes by inoculation into the milk-meat medium described by Crossley (29). Spore-forming anaerobic bacilli able to produce presumptive positive reactions in MacConkey broth were isolated frequently from certain products, e.g. pasteurized cheese, milk powder, plant swabs, etc. Similar organisms may also have been present in other samples but were not searched for unless no coliform types could be found.

For purposes of final confirmatory tests, possible coliform organisms were classed as those cultures which consisted of Gram-negative, non-spore-forming rods able to produce gas from lactose. For the latter test lactose broth containing bromo-cresol purple indicator proved useful since it sometimes yielded preliminary indication of an *aerogenes-cloacae* type. Some cultures consistently failed to produce gas in lactose broth, although in other

respects they seemed to resemble coliform types. Such doubtful strains are sometimes included in the coliform group, but were excluded in the present study.

(3) *Confirmatory tests*

The confirmatory tests now in common use were employed, viz.:

(*a*) Indol production in peptone water, using Ehrlich's reagent,

(*b*) Citrate utilization in Koser medium,

(*c*) Voges-Proskauer (O'Meara modification) and methyl-red tests in dextrose phosphate broth,

(*d*) Liquefaction of nutrient gelatin stab cultures after 4 weeks' incubation at 22° C.

In addition, the behaviour of strains in milk was studied by inoculating approx. 0·1 ml. of a 24 hr. peptone-water culture into 10 ml. separated milk containing bromo-cresol purple indicator, followed by incubation for 20 days at 37° C. and daily examination for acid production, clotting, and gas formation. Some cultures which produced slight acidity, but no clot in 20 days, were classed as coliforms if other reactions placed them in a definite coliform type. In some cases a duplicate milk tube was inoculated and then laboratory pasteurized by maintaining the milk for 30 min. at 63° C. in a thermostatically controlled water-bath; occasionally this heat-resistance test was repeated, using the same peptone-water culture after incubation for periods longer than 24 hr. (up to 5 days).

(4) *Classification*

Classification was based upon the system suggested by Wilson(1), except that the Eijkman test was not employed. Consequently the irregular types I, II and VI differentiated by Wilson have been included respectively with *coli* I, *coli* II and *aerogenes* I. The eleven types met with in this work may be summarized as follows:

Type	Indol	Citrate	M.R.	V.P.	Gelatin liquefaction
Coli I	+	−	+	−	−
Coli II	−	−	+	−	−
Intermediate I	−	+	+	−	−
Intermediate II	+	+	+	−	−
Aerogenes I	−	+	−	+	−
Aerogenes II	+	+	−	+	−
Irregular III	−	−	+	−	+
Irregular IV	−	+	+	−	+
Irregular V	−	−	−	+	−
Irregular VIII	−	−	−	−	−
Cloacae	−	+	−	+	+

The irregular type VII of Wilson was not found and type IV was only isolated once.

Low-temperature forms have not been classified separately; most of these were *aerogenes* I or *cloacae* types, but a few were classed as *aerogenes* II or intermediate II. All, therefore, were citrate-positive.

The only anomalous strain encountered was a consistently V.P.-negative form of *aerogenes* I. It was found only in milk powder and has been previously reported in another paper by Crossley & Johnson(30).

General observations on experimental methods

Before passing to detailed data, some general experimental features may conveniently be considered here.

It should be noted that most dairy products receive some form of heat treatment during

the normal processes of manufacture. Consequently, such products normally contain smaller numbers of bacteria and a less varied flora than the raw milk from which they are made; theoretically, coliform bacteria should be entirely absent from some products. As would be expected the numbers of coliform organisms as roughly estimated in this work by the normal dilution method were in general much lower in dairy products than in bulk raw milk. On theoretical grounds it might perhaps be anticipated that the incidence of a mixed coliform flora would also be lower in dairy products.

Some particular difficulties were dealt with as follows:

(1) *Overgrowth in the original sample*

Dairy products were examined almost immediately after manufacture, except in special cases, in order to avoid ascendancy of one type in the sample prior to testing, which might produce a false picture of the original flora. Some particular products, e.g. butter and hard cheese, were examined after a storage period (sometimes lengthy), and such coliform tests constituted survival tests rather than indications of the original coliform flora.

(2) *Errors of the presumptive test*

At the commencement of this study, 150 presumptive positives from various manufactured products were streaked on E.M.B. agar after 24 hr. incubation, and again from the same MacConkey broth tube after 3 days' incubation. The results agreed moderately well and seemed to indicate the presence of only one coliform strain in many of the samples. Of the 150 tubes, 123 (82%) yielded the same coliform type or types after both 24 hr. and 3 days' incubation. In twenty-one cases a mixed coliform flora was isolated from 24 hr. tubes, but only one type after 3 days' incubation; the survivors were all *coli* or intermediate I types. Conversely, in six cases additional strains (viz. two *coli*, one *aerogenes*, three irregular) were isolated from 3-day tubes which were missed in the 24 hr. tubes. Some streakings from 3-day tubes produced weakened or non-typical growths on E.M.B. agar. On balance, therefore, it seemed preferable to streak positive tubes as soon as possible.

The occurrence of a mixed coliform flora varied between different products. Of all confirmed positives examined during this study, the percentages from which more than one coliform type was isolated were distributed as follows:

Cheddar cheese 32·7, raw milk 30·5, milk powder 24·4, butter 23·8, ice cream 21·8, pasteurized cream 12·6, clotted cream 7·7, holder-pasteurized milk 6·3, soft cheese 6·0, H.T.S.T. pasteurized milk 4·1.

(3) *Observations on eosine methylene-blue agar*

This medium does not appear to have been widely used in Britain. Although in this investigation it allowed rather better differentiation of coliform types than did MacConkey agar, nevertheless it was not entirely reliable.

The presence of *coli* types alone could be recognized with fair confidence by inspection of plates, but *coli* I and *coli* II types could not be distinguished. Some *aerogenes* and *cloacae* types yielded 'typical' colony forms, but others did not, whilst even 'typical' *aerogenes* colonies were sometimes found in fact to be mixed, containing other coliform types or even non-coliform species. Although numerous intermediate and irregular types were isolated, no colony types characteristic of these groups were observed.

Furthermore, some non-coliform types (in particular closely related species such as *alcaligenes*) sometimes produced colonies bearing close resemblance to those of true coliforms. Some micrococci also grew readily on the medium and formed colonies somewhat similar to those of some *aerogenes* and irregular types; more serious trouble resulted from the fairly common occurrence of mixed coliform-*coccus* colonies which proved extremely difficult to purify. Similar difficulty has recently been reported by McKenzie *et al.* (31) during an investigation of ropy milk. Some further difficulty was experienced with a very minute 'pin-point' colony type having a strong metallic sheen. Such colonies were usually thermoduric streptococci, e.g. *Str. faecalis*, of types which occur commonly in some milk products, and were frequently associated with the presence of spore-forming anaerobes. Sometimes, however, these minute colonies yielded typical *coli* strains after replating. Some aerobic spore-forming bacilli also grew on E.M.B. agar, but created most difficulty by forming tenacious mixed colonies with coliform species. Finally, yeast colonies were also met with, but these were recognizable by inspection.

In general, the most serious obstacle was the occurrence of mixed colonies, and quite frequently the time, labour and material required to separate pure cultures greatly exceeded that involved in actual confirmatory tests. In spite of the relatively common occurrence of single coliform types in dairy products, it is difficult to visualize coliform confirmations as a regular feature of routine examinations. Such methods would be practically impossible for raw-milk samples which usually yielded both mixed and non-coliform colonies. On the other hand, it would rarely have been permissible to omit the confirmatory tests; in this respect the accepted practice of many workers appeared unsafe. Thus Yale (15) stated that *coli* and *aerogenes* types in dairy products could be recognized by inspection of colonies on E.M.B. agar.

(4) *Direct plating experiments*

Numerous attempts to estimate coliforms in milk by direct plating have been made. Yale (15) reported that for milk and cream direct plating on E.M.B. agar was satisfactory, but other workers have not supported this view. Thus Cunningham & Glathe (32) found no satisfactory plating medium and experienced difficulty with *Proteus* spp., *alcaligenes* spp., *Str. faecalis* and cocci. Kon (33) found that direct plating of raw milk on MacConkey agar yielded a large proportion of *aerogenes* and intermediate but very few *coli* colonies (the number of samples was small); coliform types could not be differentiated on MacConkey, Endo, E.M.B., or ferrocyanide-citrate agar. Wilson (1) plated raw milk on MacConkey agar but met with non-coliform colonies which were indistinguishable by naked-eye observation, nor could *coli* and *aerogenes* types be differentiated with certainty. Similar experiments with pasteurized milk failed to obtain any colonies.

In view of the obvious theoretical advantages, a preliminary study was made of direct plating methods in the examination of dairy products. A total of 122 samples was examined, comprising 27 pasteurized milk, 22 pasteurized cream, 20 clotted cream, 18 butter, 20 cheese, and 15 milk powder. Varying quantities were plated directly on E.M.B. agar, and, in addition, the normal MacConkey broth-presumptive test was carried out, followed by streaking of positives on E.M.B. agar. A parallel series of 25 samples of raw milk was examined similarly.

This work sufficed to show that the method was unsatisfactory owing to the small number of coliforms present in many samples. In some cases direct plating yielded no

coliform colonies, whilst in others the plates were rendered almost unworkable by the heavy inoculum required. Moreover, the incidence of non-coliform colonies was greater on direct plates than on plates streaked from MacConkey broth cultures. Since naked-eye differentiation was impossible, the direct-plate method increased the labour of isolating pure coliform cultures, particularly from raw milk.

The results summarized in Table 1 show that thirty dairy-product samples which were coliform positive in MacConkey broth yielded only thirteen positives by direct plating; the corresponding ratio from raw-milk samples was 25 to 19. MacConkey broth-negative samples were also negative by direct plating. Comparisons of types isolated showed quite good agreement for the dairy products but less close correspondence for raw-milk samples. The tendency of *coli* types to become predominant in MacConkey broth was evident, but direct plating completely missed a number of *coli* strains which were actually present in the samples.

Table 1. *Comparisons between preliminary enrichment and direct plating*

Cultivation in MacConkey broth followed by plating on E.M.B. agar	No. of samples	Direct plating of corresponding samples on E.M.B. agar: No. of samples					
		Coliform negative	*Coli* only	*Aerogenes* only	Intermediate or irregular only	Mixture of coliforms	Total positive samples
Manufactured products, 122 samples:							
Coliform negative	92	92	—	—	—	—	—
Coli only	14	8	5	1	—	—	6
Aerogenes only	8	4	—	4	—	—	4
Intermediate or irregular only	2	1	—	—	1	—	1
Mixture of coliforms	6	4	—	—	1	1	2
Total positive samples	30	—	—	—	—	—	13
Raw milk, 25 samples:							
Coli only	10	1	5	2	2	—	9
Aerogenes only	3	2	—	1	—	—	1
Intermediate or irregular only	2	1	—	—	1	—	1
Mixture of coliforms	10	2	—	2	—	6	8
Total positive samples	25	—	—	—	—	—	19

THE INCIDENCE OF COLIFORM ORGANISMS

The data which follow comprise a record of the incidence of presumptive positives at each stage of the manufacturing processes studied, the occurrence of false positive tests, and the detailed distribution of coliform types among the true positives, together with some preliminary observations concerning 'heat-resistant' strains.

The actual numbers of 'positive coliform tests' are only of limited interest in themselves, since such figures may vary quite considerably at any one plant at different times, and still more widely between different plants, according to the conditions of management, seasonal and other factors. The figures have been included because they indicate the particular stages of each manufacturing process wherein coliform contamination was most likely to occur, and experiences at different plants have suggested that such comparative findings are generally applicable to all plants of the same type.

RAW MILK

Coliform organisms occur very frequently in raw milk as produced at the farm and are invariably present in bulked supplies at the factory. Data recorded by previous workers (Table 2) show considerable variations in the incidence of individual coliform types. The

Table 2. *Summary of recent investigations of the incidence of coliform types*

Product	Date	Investigator	No. of cultures studied	Percentages found of main coliform groups		
				Coli	*Aerogenes* plus *cloacae*	Intermediate plus irregular
Raw milk	1930	Kline(36)	407	35·0	51·0*	14·0†
	1933	Malcolm(34)	797	54·2	25·0	21·0†
	1933	Yale(15)	91	63·0	26·0*	11·0†
	1935	Wilson(1)	245	30·2	37·5	32·4
	1937	Oeser(37)	164	50·0	34·2*	15·8
	1937	Bartram & Black(38)	310	57·0	22·0*	21·0†
	1940	Griffin & Stuart(14)	1358	16·5	58·6*	24·9
		Present study	228	55·2	30·3	14·5
Holder-pasteurized milk	1933	Yale(15)	21	57·0	10·0*	33·0†
	1935	Wilson(1)	46	41·3	23·9	34·8
	1937	Bartram & Black(38)	15	6·7	6·7*	86·7†
	1939	Messner & Canessa(39)	301	13·9	18·9	67·1†
		Present study	185	75·7	16·2	8·1
Ice cream	1933	Yale(15)	16	31·0	56·0*	13·0†
	1938	Nelson *et al.*(24)	86	30·2	62·8*	7·0†
		Present study	56	35·7	39·3	25·1
Pasteurized cream	1935	Pont(46)	128	76·5	11·0*	12·5†
		Present study	205	46·3	36·6	17·0
Butter (defective)	1932	Hammer & Yale(48)	25	Nil	88·0	12·0†
Butter (normal)	1937	Rice(49)	107	58·9	17·8	23·3
		Present study	310	46·4	43·9	9·7

* *Cloacae* strains not recorded separately from *aerogenes*. † Irregular strains not recorded separately.

discrepancies are probably due in part to different methods of differentiation and classification which have been adopted, whilst the possible influence of geographical factors cannot be overlooked. Moreover, Malcolm(34) showed that seasonal variations may be very considerable. For these reasons it was felt that no accepted general data were available, and although this investigation was concerned with dairy manufacturing processes a simultaneous study of the particular raw-milk supply seemed desirable.

Raw-milk supplies received from individual producers were sampled on delivery to the factory and were examined almost immediately in order to study the coliform flora of the milk as used for manufacture. Sampling was spread evenly over all seasons of the year, and for the purposes of this study was limited to supplies as received in individual churns. Further coliform contamination is possible in the factory immediately the raw milk is handled; such contamination from factory equipment does in fact occur most commonly from plant handling raw milk(35). Whilst sampling from weighing or bulk-storage tanks offers some obvious advantages, such samples may include coliform organisms contributed by factory tanks, pumps, and pipelines, and consequently may not yield a true picture of the raw-milk flora. On the other hand, it will be shown that coliform contamination arising from milk churns was almost negligible in the case of the particular supplies examined. It was hoped, therefore, that the procedure adopted would present a reasonably reliable general indication of the coliform flora of the raw milk as produced, and minimize any effects due to subsequent bacterial proliferation, accentuation of seasonal variations, or additional contamination of factory origin.

Of 167 presumptive positives examined, none was false positive. Mixed coliform strains were found in 30·5% of positives and a total of 228 strains was obtained for differentiation. The final classification of these strains (Tables 2, 3) agrees fairly well with

the findings of Malcolm (Table 2) in Scotland, which is of interest since Malcolm's samples were also taken from individual supplies within a few hours of milking. Of other investigations (Table 2), only that of Bartram & Black in America refers to direct churn samples. These three studies show some differences between the results obtained, but all agree very closely in recording an incidence of about 55% of *coli* types. Wilson(1), in England, found a markedly lower percentage of *coli* types and a much higher percentage of intermediates, but these figures refer to bulk tank samples and are not strictly comparable.

Table 3. *Raw milk*

	Raw milk as received		Laboratory-pasteurized samples
No. presumptive positives examined	167		28
No. false positives: Yeasts	Nil		—
Anaerobes	Nil		28 (100·0%)
No. true positives containing mixed strains	51 (30·5%)		—
Total coliform cultures	228		—
Coli I	118 (51·7%)	55·2%	—
Coli II	8 (3·5%)		—
Intermediate I	12 (5·3%)	8·4%	—
Intermediate II	7 (3·1%)		—
Aerogenes I	57 (25·0%)	30·3%	—
Aerogenes II	5 (2·2%)		—
Cloacae	7 (3·1%)		—
Irregular III	Nil	6·1%	—
Irregular V	9 (3·9%)		—
Irregular VIII	5 (2·2%)		—

Of the *coli* strains isolated, the majority were *coli* I types and *coli* II was not found commonly in raw milk as produced. Wilson(1), however, reported frequent occurrence of the *coli* II strain in raw milk and on hay and straw.

The *aerogenes-cloacae* group followed the *coli* group in order of frequency and included 30·3% of all strains isolated; this figure is rather lower than that recorded by Wilson and slightly higher than that found by Malcolm (Table 2). The majority of workers have reported frequencies between 22·0 and 37·5%. The *aerogenes* I strain comprised 25·0% of all cultures, and following *coli* I it was the commonest coliform strain found in raw milk; the *aerogenes* II (2·2% of cultures) and *cloacae* (3·1% of cultures) strains occurred much less frequently. Wilson(1) found 3·7% and Malcolm(34) 9·3% of *cloacae* strains, but other workers have not recorded these strains separately.

Intermediate and irregular strains together comprised only 14·5% of the cultures; the commonest individual types were intermediate I (5·3%) and irregular V (3·9%), whilst irregular III was not found. With one exception, other workers (Table 2) have reported frequencies between 11·0 and 24·9%. Wilson(1), however, found a much higher proportion (32·4%) and states that the intermediate I type was the commonest coliform organism to be isolated from hay and it was also very common in raw milk.

For the purposes of this investigation the distribution of the main coliform groups in the raw milk as received for manufacture was recorded as: *coli* group 55·2%, *aerogenes-cloacae* group 30·3%, intermediate-irregular group 14·5%, a ratio of approximately 3·7 : 2 : 1. This broad grouping has been adopted for general comparative purposes, since few previous investigators have differentiated between *aerogenes* and *cloacae* or intermediate and irregular strains.

Raw-milk plant

The coliform flora of milk churns and factory plant handling raw milk may be considered here. Washed churns were examined by the standard churn rinse technique (Min. Agric. Form No. 168/T.P.Y.), and the presumptive coliform test was carried out on 10 ml. portions of the rinse liquid. Factory plant surfaces were examined by a swab technique (Min. Agric. Form No. C195/T.P.Y.); fuller details of this work have been recorded elsewhere by Crossley(35), but the coliform tests described here were performed with 10 ml. quantities of the swab solutions.

The data presented in Table 4 show that churns washed by mechanical churn-washers contained very few coliform organisms, even when large quantities of rinse liquid were examined, since 1006 churns yielded only 3·7 % of presumptive positives. Churns washed by hand, however, yielded 47·6 % of presumptive positives, but none of the raw-milk supplies examined was delivered in hand-washed churns. Furthermore, confirmatory tests revealed that 45·4 % of the presumptive positives were actually false positives. Of the remaining true positives, a high proportion (41·7 %) contained mixed coliform strains. The incidence of all *coli* strains (53·0 %) was very similar to that found in raw milk, but the *coli* II strain was much commoner than in raw milk. The *aerogenes-cloacae* group (17·6 %) was less common than in raw milk, but intermediate (14·7 %) and irregular (14·7 %) types were much more frequent. These findings are probably related to the heat resistance of coliform strains, which will be discussed later. It may be noted here that unsatisfactory churn-washing may increase the proportion of intermediate, irregular, and *coli* II strains present in raw milk.

The plant swab data (Table 4) show that factory-plant surfaces may quite frequently contribute additional coliform organisms to raw milk. This is due to the heavy initial infection of such surfaces and the difficulty of applying adequate heat sterilization to large plants. The number of confirmatory tests was not large, but the results tended to indicate a high proportion of false positive reactions, whilst the intermediate-irregular group occurred more frequently than in raw milk. Bulk samples of raw milk which has contacted tanks, strainers, pumps, and pipelines may possibly contain a higher proportion of intermediate-irregular strains than may be present in the original raw milk; this possibility is supported by the findings of Wilson(1), who suggested, however, that the high proportion of intermediate strains found in his bulk raw-milk samples originated directly or indirectly from soil contamination in the form of dust.

Pasteurized milk

The coliform flora of pasteurized milk has received rather less attention than that of raw milk, and in general the number of confirmatory tests reported has not been large. The summarized findings of some previous workers (Table 2) show pronounced variations, and this investigation has revealed a number of factors which may affect any attempt to record the incidence of coliform strains.

(*a*) *Holder process*

Recorded findings of previous workers mainly refer to the holder process of pasteurization. The complete process of producing bottled pasteurized milk comprises several distinct operations, and bacteriological data must be related to the point of sampling.

Table 4. *Dairy plant*

Incidence of presumptive positive tests from 10 *ml. inoculations*

	Total tests	Presumptive positives		Presumptive positives	
		No.	%	No.	%

Table 4. *Dairy plant*

Incidence of presumptive positive tests from 10 ml. inoculations

Plant washing water, various:	Total tests	Presumptive positives No.	Presumptive positives %
All samples	68	50	73·5
Plant rinse waters:			
Pipelines	200	5	2·5
Holder and open capillary cooler	314	26	8·3
Holder and enclosed cooler	56	2	3·6
Heat exchangers, plate type	96	1	1·0
Pasteurized milk pumps	18	1	5·5
Separators	16	14	87·5
Milk evaporators, climbing film type	12	Nil	Nil
Cheese tanks, before steaming	14	10	71·4
Churn rinses:			
Mechanical churn-washers (mean of 4 machines)	1006	37	3·7
Hand-washed churns	63	30	47·6

Plant swabs:	Total tests	Presumptive positives No.	Presumptive positives %
Raw milk:			
Tanks and pipelines	131	20	15·3
Pumps	10	3	30·0
Filters and strainers	36	8	22·2
Coolers	11	2	18·2
Pasteurized milk:			
Heating tubes	8	Nil	Nil
Hot-milk distributing pipes	10	Nil	Nil
Holder compartments	10	Nil	Nil
Holder outlet valves	28	Nil	Nil
Coolers	96	6	6·2
Pipelines and balance tanks including agitators	61	11	18·0
Butter:			
Cream coolers	12	Nil	Nil
Cream storage vats	8	2	25·0
Butter churn and worker	115	10	8·7
Cheese:			
Tanks	12	4	33·3
Evaporator and spray drying plant:			
Vapour separators	4	Nil	—
Evaporator discharge tank	20	2	10·0
Atomizer feed tank	20	3	15·0

Incidence of coliform types

Plant swabs:	No. presumptive positives examined	False positives anaerobes	No. true positives containing mixed strains	Total coliform cultures	*Coli* I	*Coli* II	Intermediate I	Intermediate II	*Aerogenes* I	*Aerogenes* II	*Cloacae*	Irregular III	Irregular V	Irregular VIII
Bulk raw-milk tanks and pipelines	17	6	2	13	4	2	2	1	3	Nil	Nil	Nil	1	Nil
Filters and strainers	8	5	Nil	3	1	Nil	1	Nil	1	Nil	Nil	Nil	Nil	Nil
Pasteurized milk coolers	6	4	Nil	2	1	Nil	Nil	Nil	Nil	1	Nil	Nil	Nil	Nil
Pasteurized milk balance tanks and pipelines	9	3	Nil	6	3	1	1	1	Nil	Nil	Nil	Nil	Nil	Nil
Butter churn and worker	10	1	5	16	5	Nil	4	Nil	5	1	1	Nil	Nil	Nil
Evaporator plant	5	Nil	5	10	4	1	2	Nil	3	Nil	Nil	Nil	Nil	Nil
Totals	55	19	12	50	22		12		15			1		
		34·5%	33·3%		44·0%		24·0%		30·0%			2·0%		
Plant washing water	46	3	3	46	23	4	1	Nil	14	2	Nil	Nil	Nil	2
Totals		6·5%	7·0%		27		1		16			2		
					58·7%		2·2%		34·8%			4·3%		
Plant rinse waters:														
Pipelines	5	Nil	1	6	5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	1	Nil
Holder and cooler	22	1	4	25	19	Nil	Nil	Nil	4	Nil	Nil	Nil	Nil	2
Separator	14	Nil	3	17	12	Nil	Nil	Nil	3	1	Nil	Nil	1	Nil
Totals	41	1	8	48	36		Nil		8			4		
		2·4%	20·0%		75·0%				16·7%			8·3%		
Churn rinses	44	20	10	34	11	7	5	Nil	4	2	Nil	1	2	2
Totals		45·4%	41·7%		18		5		6			5		
					53·0%		14·7%		17·6%			14·7%		

Coliform contamination may arise from the presence of heat-resistant strains in the raw milk, from inefficient pasteurization, from pasteurizing plant contamination, from bottling plant contamination, and from the bottles themselves. In this investigation the process of pasteurization proper has been considered to include the operations of heating, holding, cooling and storage. These operations must be carried out whether the pasteurized milk is subsequently bottled or dispatched in churns or other containers, or used for manufacture of other products. The examination of bottled pasteurized milk reveals the sum total of contamination from all sources, but includes contamination derived from bottles and bottling machinery which is not strictly related to pasteurizing plant contamination. In particular, the study of pasteurized milk utilized for dairy manufacture must exclude the bottling process. Strict comparisons between the findings of previous workers are probably of doubtful value. The nineteen positives studied by Yale (15) comprised thirteen from bottles and six from holder compartments (three operating below the designated temperature); the forty-six cultures classified by Wilson (1) were isolated from bottled milk, whilst the exact source of the fifteen cultures isolated by Bartram & Black (38) is not stated. The 301 cultures studied by Messner & Canessa (39) were obtained from both holder- and flash-pasteurized milk.

Apart from failure to maintain the correct holding temperature or time, inefficient pasteurization may be due to the presence of excessive froth in holder compartments, to leakages of raw milk into the pasteurized milk, and to inadequately heated pockets in the plant. Recontamination of the pasteurized milk may be derived from coolers, pumps, pipelines and balance tanks. Data derived from the study of three holder plants are summarized in Table 5.

Plant 1 incorporated a holder of unsatisfactory design wherein valve leakages were not diverted, so that slight leakages of raw milk into holder compartments, and of partly held hot milk direct to the cooler, were known to occur. The valve pockets were situated outside the plant. Considerable froth accumulated in the holder and cooling was by a large, open, capillary cooler. Samples of milk as discharged from the holder compartments yielded 7·6% of presumptive positives, whilst 14·3% of presumptive positives were obtained from holder froth, although the holding times and temperatures had been maintained correctly. Valve leakages passing to the cooler yielded 75·0% of presumptive positives, but these were intermittent and difficult to sample. The most serious coliform contamination, however, was derived from the cooler, at which point the incidence of positive tests rose to 20·0% at the top surface and 35·3% after passage of the milk over the whole surface.

Plant 2 comprised a rotating-type holder of good design, in which raw-milk contamination was impossible and all valve leakages were definitely diverted. Coliform tests of direct holder samples were all negative. The use of an open, capillary type cooler again resulted in the occurrence of 28·3% of positive tests from the cooled milk.

Plant 3 was a very large, modern type of recent design, and included enclosed capillary coolers built in leaf sections to facilitate cleaning and sterilization. Direct holder discharge samples yielded only one presumptive positive (a false positive). Cooler samples were more satisfactory than those obtained from plants 1 and 2, but still showed an incidence of 9·0% presumptive positives, although some of these were not true positives. In this plant the main coliform contamination was derived from balance tanks, pumps, pipelines, and churn-filling devices handling the pasteurized milk.

Table 5. *Pasteurized milk*

Incidence of presumptive positive tests

Table 5. *Pasteurized milk*

Incidence of presumptive positive tests

Holder process	Total tests	Presumptive positive tests: No.	Presumptive positive tests: %
(1) Old type holder, no diversion of valve leakages, froth in holder compartments. Large open capillary cooler:			
(*a*) Holder discharge	92	7	7·6
(*b*) Froth in holder	7	1	14·3
(*c*) Valve leakages passing to cooler	8	6	75·0
(*d*) Milk from top of cooler	70	14	20·0
(*e*) Milk from bottom of cooler	136	48	35·3
(*f*) Milk from churns	2213	844	38·1
(2) Rotating type holder, no raw-milk contamination, valve leakages diverted. Large open capillary cooler:			
(*a*) Holder discharge	40	Nil	Nil
(*b*) Milk from bottom of cooler	92	26	28·2
(3) Large modern plant, valve leakages diverted. Enclosed capillary coolers in small sections:			
(*a*) Holder discharge	108	1	0·9
(*b*) Milk from coolers	412	37	9·0
(*c*) Milk from balance tanks	1664	436	26·2
(*d*) Milk from churns	2746	895	32·6
(4) Milk pasteurized in sealed bottle	35	3	8·6

High temperature-short time process	Total tests	Presumptive positive tests: No.	Presumptive positive tests: %
(5) Old type 'scalder', large open capillary cooler:			
(*a*) Heater discharge	22	2	9·1
(*b*) Milk from cooler	54	30	55·5
(*c*) Milk from storage tanks	14	13	92·9
(*d*) Milk from churns	13	12	92·3
(6) Modern tubular heater, large open capillary cooler:			
(*a*) Heater discharge	55	3	5·4
(*b*) Milk from cooler	56	38	67·8
(*c*) Milk from storage tanks	44	34	77·3
(7) Modern plate type heater and cooler. Flow diversion valve fitted:			
Discharge from cooling section	168	8	4·8

Incidence of coliform types

	No. presumptive positives examined	False positives anaerobes: No.	False positives anaerobes: %	No. true positives containing mixed strains	Total coliform cultures	*Coli*: I	*Coli*: II	Intermediate: I	Intermediate: II	*Aerogenes*: I	*Aerogenes*: II	*Cloacae*	Irregular: III	Irregular: V	Irregular: VIII
Holder process:															
Holder discharge	13	6	46·1	1	8	4	1	—	—	3	—	—	—	—	—
Holder froth	4	2	50·0	Nil	2	1	—	—	—	—	—	—	—	1	—
Cooler	29	5	17·2	1	25	21	—	—	—	3	—	—	—	—	1
Balance tanks and churns	171	30	17·5	9	150	93	20	6	1	17	1	6	1	3	2
Totals	217	43	19·8	11	185	119	21	6	1	23	1	6	1	4	3
				6·3%		75·7%		3·8%		16·2%			4·3%		
H.T.S.T. process	49	3	6·1	2	48	20	5	2	Nil	14	2	2	2	Nil	1
				4·1%		52·1%		4·2%		37·5%			6·2%		

Comparisons between old- and modern-type holder plants (%)		False positives	True positives: *Coli*	True positives: Intermediate	True positives: *Aerogenes-cloacae*	True positives: Irregular
	Old	13·4	75·2	1·4	19·3	4·2
	Modern	36·7	77·5	12·5	5·0	5·0

Adequate heat sterilization of very large plants has been found difficult and uncertain, whether attempted by means of steaming or circulating hot-water rinses. The use of hypochlorite sterilization, recently legalized, has greatly facilitated the elimination of coliform organisms. The introduction of hypochlorite rinses as an addition to the normal steam and hot-water treatment of plant 3 yielded greatly improved results, which may be quoted. Of 4160 samples of pasteurized milk taken from a balance tank prior to the use of hypochlorite 1331 (32·0%) yielded positive presumptive tests, but when hypochlorite was used 655 similar samples yielded 67 (10·2%) presumptive positives. No doubt the incidence of coliforms from plants 1 and 2 would also have been lower had hypochlorite treatment been possible at the time when these plants were in use.

It should be stated that the actual numbers of coliform organisms were small, since positive tests were rarely obtained from less than 1 ml. of milk, and in a number of cases 10 ml. inoculations were required to obtain positives. Nevertheless, it is clear that coliform organisms are not readily eliminated and the presumptive positive test is now well recognized to be one of the most delicate means of detecting slight recontamination of pasteurized milk. (The holder samples taken from plants 2 and 3 showed that the possibility of positive coliform tests due to heat-resistant strains present in the raw milk was negligible, and other evidence supporting this conclusion will be discussed.)

A small number of samples pasteurized by the 'In-bottle process' (plant 4) were examined; in this process recontamination is impossible. The three presumptive positives were obtained from milk which had entered the hot-air holding chamber at 141 instead of 145° F. The survival of coliform organisms, and positive phosphatase tests, both indicated that the initial loss of temperature was not recovered in the hot-air chamber maintained at 145° F.

High temperature-short time process

Until recently this process has been used less extensively than the holder process, and few results relating to plants of recent design have been published. Three plants were studied during this investigation (Table 5).

Plant 5 employed a 'scalder'-type heater, now obsolete, which yielded in effect a mixture of overheated and underheated milk. Positive coliform tests were obtained from 9·1% of samples taken directly from the discharge of this heater, which operated at 163–165° F.

Plant 6 comprised a tubular heater of modern design, wherein the milk was heated at 163–165° F. for 20 sec. Hiscox(40) has recently reported an investigation of the H.T.S.T. process and mentions the difficulties associated with the uniform heating of liquids in continuous flow through tubes. Samples taken directly from the tubular heater discharge yielded 5·4% coliform positives; these results support the view that small quantities of underheated milk may pass through such heaters.

Plants 5 and 6 both employed very large, open capillary coolers and large storage tanks for the pasteurized milk. In both cases coliform contamination from these sources was very heavy.

Plant 7 comprised a modern plate-type heater and cooler, fitted with adequate temperature control instruments and a flow diversion device designed to divert underheated milk. Samples of milk leaving the cooling section yielded only 4·8% coliform-positive tests, all of which were obtained from 10 ml. quantities of milk. These positives would include coliforms derived from both underheated milk (if any) and recontamination from the cooling section, and it will be observed that in respect of freedom from coliform contami-

nation the performance of this plant was better than that of any of the holder plants. The plant contained no components of large size, and the design facilitated efficient cleaning and sterilization.

Coliform strains

False positive reactions due to anaerobes comprised about 50% of the presumptive positives obtained from holder samples (Table 5). Such results might be expected (especially from 10 ml. inoculations), since only heat-resistant organisms would be anticipated in direct holder samples, but obviously a positive presumptive test alone cannot provide definite proof of the presence of coliform organisms. Cooler and balance tank samples included coliform recontamination and showed a much lower, though not negligible, proportion (17%) of false positives. Only 6·1% false positives were obtained from H.T.S.T. plant.

Only 6·3% of holder plant true positives and 4·1% of H.T.S.T. plant true positives contained mixed coliform strains—a further indication of the small number of coliforms in pasteurized milk.

Of the coliform cultures associated with the holder process, 75·7% belonged to the *coli* group, which was clearly predominant. The *aerogenes* and *cloacae* strains were much less common, comprising 13·0 and 3·2% of the cultures, whilst intermediate and irregular strains each comprised about 4% of the cultures. The *coli* strains were therefore more frequent, and the *aerogenes*, intermediate and irregular strains less frequent than in raw milk. It may be noted that the *coli* II strain comprised 15% of the *coli* strains from pasteurized milk, from which, contrary to the findings of Wilson(1), it was isolated more commonly than from raw milk.

Separate data for plants 1 and 3 reveal interesting comparisons (Table 5). The percentage of *coli* and irregular strains was almost identical from both plants, but the modern plant yielded a much lower percentage of *aerogenes-cloacae* strains and a much higher percentage of intermediates than did the old plant. Moreover, the modern plant yielded 36·7% false positives as compared with 13·4% from the old plant.

Cultures associated with the H.T.S.T. process showed a much lower proportion of *coli* strains (52·1%), an approximately similar proportion of intermediate (4·2%) and irregular (6·2%) strains, and a much higher proportion of *aerogenes* (33·3%) and *cloacae* (4·2%) strains than did holder process cultures.

Heat-resistance tests showed that 34·6% of the pasteurized milk strains examined survived laboratory pasteurization in milk at 145° F. for 30 min.; *coli* I and irregular strains survived most frequently. Wilson(1) also found 30·0% of heat-resistant strains, mainly the *coli* I type, and concludes that heat-resistant *coli* strains in raw or pasteurized milk are probably of excretal origin. American workers have frequently reported such strains and appear to assume that the organisms must have survived the pasteurizing process; on these grounds they conclude that the coliform test is of little value in the control of pasteurized milk.

Whilst the possibility that some coliform organisms may survive the pasteurization process cannot be overlooked, it is suggested that this explanation of the presence of heat-resistant strains in pasteurized milk is inadequate except in a limited number of cases.

In the first place, heat-resistant coliforms appear to be uncommon in raw milk produced in Britain. In this investigation 9% of the coliform organisms isolated from raw milk were heat resistant, whilst Wilson(1) found 5% of resistant strains, but it would appear

that such figures may only apply to a small number of cells in laboratory cultures. Direct laboratory pasteurization of 498 samples of raw milk as received from individual producers yielded no heat-resistant coliforms—seven presumptive positives were obtained from 10 ml. inoculations, but all were false positives. A further 484 bulked tank samples of raw milk were also laboratory pasteurized and yielded seventeen (3·5%) presumptive positives, of which only two (0·2%) were true positives. Vernon & Walker(22) also failed to isolate any heat-resistant coliforms from 440 samples of bulked raw milk, but were able to obtain thirty-three heat-resistant cultures from 1000 presumptive positives from pasteurizing plant. Moreover, it may be significant that in this investigation 33·3% of the coliform cultures isolated from churn rinses and 18·8% from swabs of raw-milk plant were heat resistant (Table 15). It is therefore suggested that heat-resistant coliform organisms occur in very small numbers in raw milk, and that the major source of these may well be churns and factory equipment used in the reception and bulking of raw milk, i.e. within the factory rather than on the farm. Except during severe winter weather the organisms present in raw milk at the time of delivery to the factory are in a state of vigorous proliferation when they would be most susceptible to destruction by heat.

Secondly, direct holder samples from plants in which underheating of the milk was prevented contained few or no coliform organisms, which would be expected in view of the very infrequent occurrence of heat-resistant coliforms in the raw milk. At the same time, a very large proportion of the coliform organisms isolated from the cooling and other post-heating processes were in fact heat resistant, and if these organisms had survived the heating process it seems difficult to account for their absence from holder samples. Errors of pasteurization such as slight underheating or the presence of froth might well result in the selective survival of the most heat-resistant coliforms present in the milk supply, but this explanation alone does not entirely account for the facts. The plants in which such errors were prevented (as confirmed by phosphatase tests) yielded the highest proportion of heat-resistant coliforms, whilst when these errors were known to occur there was a marked rise in the incidence of non-heat-resistant *aerogenes-cloacae* types. It would appear, therefore, that the main source of the heat-resistant strains is the same as that of all coliforms in general, viz. recontamination after heating.

Two other findings support this possible explanation. Close observation of pasteurizing plants for several years has often revealed the occurrence of particular coliform strains over a period of several consecutive days, followed by a long period of absence; a specific plant infection seems to be the most likely explanation of such findings.

The coliform flora of dairy plants, as revealed by swab methods, has also been studied. Plant swabs yielded few presumptive positives from 10 ml. inoculations, and the number of coliform cultures available has not been very large (Table 4). It is significant, however, that swab cultures from raw-milk plants yielded 18·8% heat-resistant strains, but 50·0% of the coliform cultures swabbed from pasteurizing plants were heat resistant (Table 15). The cleaning of milk plants has an important bearing upon this problem. Some portions at least of most large dairy plants must be cleaned by manual brushing of surfaces with hot water or detergent solutions. Such washing waters are frequently contained in tanks and may readily become contaminated by manual contact, by brushes or cloths, or by organisms removed from many different items of equipment. Bacteriological examinations have shown that large numbers of organisms, including coliforms, may sometimes be found in plant washing waters when running water is not used (Table 4). The necessary

operation of physical cleaning may therefore *increase* the bacteriological contamination of plants and introduce a coliform infection which was not originally present. In this respect washing waters used for raw-milk equipment are the most dangerous. Of thirty-one coliform cultures obtained from washing waters 54·8% were heat resistant; a selective accumulation of resistant cells would be expected, since washing waters are hot and may also contain alkaline detergents. Sterilization of the plant before use is therefore essential and for this purpose flushing with very hot water rinses is commonly used. Unless large volumes of hot rinse are used, some coliform organisms present on large items of equipment may survive destruction, in particular the heat-resistant types. Examinations of hot rinses leaving pasteurizing plants did not often reveal the presence of coliforms (Table 4), but of thirty-four coliform cultures collected from such sources over a long period 64·7% were heat resistant (Table 15).

A tentative explanation of the coliform flora of pasteurizing plants may be suggested on ecological lines. The *coli* I type is the typical coliform of holder-pasteurizing plants because the particular conditions favour this strain. The complete heat-resistance data (Table 15) may be anticipated here to state that the most resistant strains appear to be *coli* I and intermediate II (which is uncommon in both raw and pasteurized milk). During the operating period (usually several hours) considerable portions of holder-pasteurizing plants are maintained at high temperatures which almost certainly eliminate heat-susceptible coliforms. At the conclusion of the pasteurizing run all portions of the plant are cleaned with hot washing waters, in which the majority of any coliforms present are heat resistant. Finally, the plant receives some form of sterilizing treatment, and any coliform survivors of this process are most likely to be resistant strains. Consequently, any residual coliform infection remaining in the plant comprises a large proportion of heat-resistant strains, and the recurrence of this cycle over several consecutive days may result in the accumulation of a considerable plant infection. In practice it has been observed that coliform positive samples of pasteurized milk occurred in spells of several days, followed usually by long periods of absence. Any coliform organisms present in the raw-milk supply which subsequently survive pasteurization are likely to be mainly heat-resistant *coli* I strains; such organisms would obviously be a serious source of plant contamination, but it is believed that normally the number of such survivors is extremely small. There appears to be little direct relationship between the coliform floras of raw and pasteurized milk, but there may be indirect contact via common operatives, cleaning waters, brushes, etc., if employed in cleaning both raw and pasteurized milk equipment. When the pasteurization process itself is inefficient (owing to underheating, leakages of underheated milk in holder plants or passage of underheated milk through H.T.S.T. plants), or the cleaning and sterilizing processes are markedly inadequate, the coliform flora of pasteurized milk appears to include a higher proportion of *aerogenes-cloacae* types. If this observation is generally true the hygienic significance of the *aerogenes-cloacae* types is greater than that of the *coli* types in the particular case of pasteurized milk.

Considerable attention was devoted to the coliform flora of pasteurized milk since pasteurized rather than raw milk is the normal raw material of other dairy products. Vernon & Walker (22) state that the various processes involved in the bottling of pasteurized milk may be serious sources of coliform contamination; similar observations have been made during this investigation, but at present few results of confirmatory tests are available. It may be noted, however, that eighteen presumptive positives obtained from

rinses of washed and 'sterilized' milk bottles ready for filling yielded 1 anaerobe, 1 *coli* I, 1 *coli* II, 8 intermediate I, 3 intermediate II, 2 *aerogenes* I, and 2 *cloacae* cultures. It seems possible, therefore, that contaminated bottles are an important source of intermediate strains and probably of *aerogenes-cloacae* strains, and this explanation may account for the high proportion of intermediate cultures isolated by other workers from bottled pasteurized milk.

Ice cream

Large-scale ice-cream manufacture is somewhat similar to the pasteurization of milk by the holder process, but differs in respect of the raw materials, which may include various combinations of milk, cream, dried milk, sweetened condensed milk and butter. Additional non-dairy ingredients are also used, viz. sugar, gelatin, various gums and possibly cornflour or eggs. If the mix is compounded entirely from butter, milk powder and condensed milk the raw materials may on occasion be free from coliform organisms. The ingredients are heated together with agitation in a batch cooker; the heating temperature employed may vary between 145 and 200° F., and heating times are also variable. The hot mix is homogenized and then cooled either by passage over an open-type capillary cooler or by passage through an enclosed plate-type heat exchanger. The cooled mix is held in storage tanks to await freezing, which may be carried out by the bulk manufacturer. Alternatively, the ice-cream mix may be distributed in liquid, condensed, or dried form to small-scale operators, who carry out the operations of freezing and retail distribution.

Public authorities in Britain have commented unfavourably upon the frequent occurrence of coliform organisms in ice cream, as revealed by the presumptive test. In America, Yale(15) and Nelson *et al.*(24) have isolated and classified coliform types (Table 2). The latter workers showed that twelve *coli* cultures which were killed by heating in separated milk at 142° F. for 30 min. survived similar treatment in ice-cream mix, but later Nelson Caulfield & Martin(41) reported that these types were destroyed more readily than phosphatase. Paley & Isaacs(25) found that the heat resistance (at 143° F.) of *coli* in ice-cream mix was double that in milk, and attributed the difference to a protective action of stabilizer gums. In Britain, however, such considerations are less important, since ice-cream mixes are usually processed at temperatures higher than 145° F.

Data obtained from the study of coliform organisms associated with the large-scale manufacture of bulk ice-cream mix are summarized in Table 6. Of the unheated mixes 80% gave positive coliform tests, but no positive tests were obtained from samples of the same mixes after heating, and in view of the temperatures employed the survival of coliform organisms was much less likely than in pasteurized milk. The presence of coliform organisms in ice-cream mixes was due to recontamination after heating. Some contamination, probably slight, was derived from the homogenizer, but lack of adequate sterilization of open-type surface coolers was undoubtedly the major cause of contamination at this particular plant. In contrast, no coliform organisms were obtained from ice cream cooled by means of a plate-type cooler.

The coliform flora differed considerably from that found in pasteurized milk. No false positives were obtained from ice cream, whilst a high proportion (21·8%) of the true positives contained mixed coliform strains. The *aerogenes-cloacae* group (39·3% of cultures) occurred most frequently, closely followed by the *coli* group (35·7%). In comparison with pasteurized milk cultures, therefore, the *coli* types were much less common, whilst the irregular types were slightly more frequent and intermediate types much more frequent.

The incidence of *aerogenes* types was about the same as in H.T.S.T. pasteurized milk, but much higher than in holder-pasteurized milk. These findings may be attributed to inadequate sterilization of the cooling plant from which most of the cultures were derived. In addition, the predominance of *aerogenes* types may be characteristic of dairy products containing added sucrose, since these types were also the commonest coliforms found in sweetened condensed milk. Only 15·8% of the coliform cultures isolated from ice cream were heat resistant, which would be expected in view of the relatively low proportion of *coli* types.

Under some circumstances additional coliform contamination of ice cream may occur during the operations of freezing and retail distribution, but extensive investigation of these processes was not possible.

Table 6. *Ice cream*

Incidence of presumptive positive tests

	Total tests	Presumptive positive tests	
		No.	%
Mix before heating	20	16	80·0
Mix after heating	20	Nil	Nil
Mix after homogenization	14	2	14·3
Mix after cooling by plate-type heat exchanger	14	Nil	Nil
Mix after cooling by large open capillary cooler	20	16	80·0
Finished mix in storage vessels	103	55	53·4

Incidence of coliform types

	No.	%	
No. presumptive positives examined	46	—	
False positives: Yeasts	Nil	—	
Anaerobes	Nil	—	
No. true positives containing mixed strains	10	21·8	
Total coliform cultures	56	—	
Coli I	18	32·1	35·7
Coli II	2	3·6	
Intermediate I	8	14·3	17·9
Intermediate II	2	3·6	
Aerogenes I	20	35·7	39·3
Aerogenes II	2	3·6	
Cloacae	Nil	—	
Irregular III	Nil	—	7·2
Irregular V	2	3·6	
Irregular VIII	2	3·6	

DRIED MILK

The coliform flora of spray-dried milk has been fully discussed in a previous paper by Crossley & Johnson (30). Since that time additional data have been accumulated which substantially confirm the original conclusions, whilst new data have been obtained concerning the effects of high-temperature pre-heating of milk destined for spray drying. Samples were taken at two large Kestner-type evaporating and spray-drying plants, of which plant 1 handled a raw-milk supply of poor bacteriological quality, whilst plant 2 handled a better raw-milk supply, and in addition employed more effective methods of plant cleaning and sterilization.

Pre-heating of the raw milk was carried out by a H.T.S.T. process, using modern tubular-type heaters. Following pre-heating the hot milk was concentrated to 40–45% solids by

passage in continuous flow through a triple circulation climbing film evaporator operating under high vacuum. Owing to the large size and intricate construction of the evaporating plant, and the accumulation of concentrated milk deposits, adequate cleaning and sterilization was not easy (30). The warm concentrated milk (at about 100–110° F.) was delivered by pump and pipelines to storage (atomizer) tanks feeding the atomizer of the spray-drying plant.

Prior to 1942 the pre-heating process was performed at 163–165° F. for 20 sec., and sterilization of the plant was carried out by a combination of steam treatment and hot-water rinses. During investigations carried out in 1942–3 by Mattick, Hiscox, Crossley *et al.* (42) and Crossley (43) the pre-heating treatment was increased to 190° F. for 20 sec., and sterilization of the whole plant was greatly improved by increasing the efficiency and extent of the steam treatment employed and by additional flushing with hypochlorite rinses.

During the period of low-temperature pre-heating, presumptive positive tests (Table 7) were obtained from 19·5% of pre-heated milks from the heater discharge at plant 1 and 7·6% at plant 2. The positive tests were only obtained from 20 ml. quantities of pre-heated milk and the figures are rather deceptive, since nine of twenty-one presumptive positives examined were not true positives. Moreover, 64% of the true positives were heat-resistant strains. Nevertheless, it was apparent that slight temperature fluctuations did occur and that the margin of safety was insufficient; any coliform survivors passed into the evaporating plant. Considerable coliform contamination occurred in the evaporator, and concentrated milks from the evaporator discharge yielded 75·7% presumptive positives at plant 1 and 56·0% at plant 2. The coliform test was an excellent indication of the efficiency of plant sterilization, and positive reactions were obtained in quantities varying from 10 to 0·001 ml. of concentrated milk. Pumps, pipelines, and atomizer feed tanks frequently contributed additional coliform organisms; concentrated milks entering the drying plant yielded 88·5% presumptive positives at plant 1 and 61·0% at plant 2.

A considerable decrease occurred during the period of high-temperature pre-heating and improved plant sterilization. No presumptive positives were obtained from pre-heated milks (Table 7). Concentrated milks from the evaporator yielded 34·4% positives at plant 1 and 6·0% at plant 2, whilst atomizer feed-tank samples yielded 59·1% positives at plant 1 and 10·9% at plant 2. Moreover, the positive reactions were usually obtained only from 1 ml. inoculations of concentrated milk (equivalent to 5 ml. normal milk).

Differential tests (Table 7) showed that *coli* I predominated throughout the pre-drying stages, although some progressive recontamination by more varied types (mainly *aerogenes* I) occurred throughout the evaporator stages. The process temperatures (which were maintained for many hours) tended throughout to favour *coli* types but probably permitted at least survival of other types in the concentrated milk. The cultures isolated from pre-heated milks were largely *coli* types and 64% were heat resistant. During its passage through the evaporator the milk cooled from pre-heating temperature to about 110° F., and 59% of cultures from the evaporator discharge were heat resistant. Subsequently the concentrated milk cooled to 90–100° F. in the atomizer feed tank, and 50% of the coliform cultures obtained from this sampling point were heat resistant.

Many bacteria, including some coliform organisms, fail to survive the spray-drying process, and consequently very few coliforms are found in spray-dried milk. Inoculations of 20 ml. reconstituted milk were commonly required to demonstrate the possible presence

Table 7. *Spray process milk powder*

Incidence of presumptive positive tests

	Plant 1			Plant 2		
		Presumptive positives			Presumptive positives	
	Total samples	No.	%	Total samples	No.	%
Milk preheated at 163–170° F. No hypochlorite treatment of plant						
Preheated milk	41	8	19·5	264	20	7·6
Concentrated milk leaving evaporator	33	25	75·7	116	65	56·0
Concentrated milk entering atomizer	26	23	88·5	77	47	61·0
Milk powder	442	132	29·8	200	43	21·5
Milk preheated at 188–190° F. Plant given hypochlorite treatment						
Preheated milk	22	Nil	Nil	184	Nil	Nil
Concentrated milk leaving evaporator	22	8	36·4	184	11	6·0
Concentrated milk entering atomizer	22	13	59·1	184	20	10·9
Milk powder	543	138	25·4	570	53	9·3
All powder samples, both plants	1755	366	20·8	—	—	—

Incidence of coliform types

	Pre-heated milk	Concentrated milk leaving evaporator	Concentrated milk entering atomizer	Total all pre-drying stages		Milk powder	
No. presumptive positives examined	21	33	34	88		366	
False positives, anaerobes	9	Nil	1	10 (11·4%)		190 (51·9%)	
No. true positives containing mixed strains	1	6	4	11 (14·1%)		43 (24·4%)	
Total coliform cultures	13	39	37	89		219	
Coli I	8	26	22	56	67·4%	75	37·9%
Coli II	2	1	1	4		8	
Intermediate I	—	—	3	3	6·7%	5	3·6%
Intermediate II	—	2	1	3		3	
Aerogenes I	2	6	8	16	20·2%	72	55·3%
Aerogenes II	—	—	—	—		2	
Cloacae	—	1	1	2		47	
Irregular III	—	—	—	—	5·6%	1	3·2%
Irregular V	1	2	—	3		3	
Irregular VIII	—	1	1	2		3	

of coliform organisms, and no presumptive positives were obtained from less than 10 ml. The incidence of presumptive positives in 20 ml. reconstituted milk (approx. 2 g. powder) varied from 29·8% at plant 1 employing low-temperature pre-heating, down to 9·3% at plant 2 when high-temperature pre-heating was operated. The true incidence of coliform organisms was actually much lower. Owing to the heavy inoculations required the incidence of false positive tests was very high and amounted to 52% of all the presumptive positives.

Differential tests (Table 7) revealed pronounced changes in the coliform flora following spray drying. The *coli* group showed a marked decrease, whilst the *aerogenes-cloacae* group showed a pronounced increase. (It should be observed that powder samples were taken directly from the drier discharge into sterile bottles, so that no recontamination occurred.) In contrast to the predominance of *coli* I prior to drying, the *coli* I and *aerogenes* I strains were found in almost equal numbers in dried milk, whilst the *cloacae* strains comprised a much larger proportion of the coliform flora after spray drying and included 21·5% of all the cultures isolated from spray-dried milk. The unusual frequency of *cloacae*

strains was an outstanding feature of the coliform flora of dried milk. The intermediate-irregular types were not common at any stage and were probably proportionately decreased by drying.

It is interesting to note that of seventy-two coliform cultures isolated from dried milk which were subsequently laboratory pasteurized in milk, only 32% were heat resistant—a lower proportion than at any pre-drying stage. The heat-resistant cultures included some *aerogenes* and *cloacae* types, but the majority of these were killed. The data seem to suggest that the *aerogenes-cloacae* group is more resistant to spray drying than the *coli* group, and that organisms which fail to resist pasteurization in milk may survive spray drying, and vice versa. This combination of circumstances is probably fortunate in that the coliform types of most frequent occurrence throughout the pre-drying stages may be the most vulnerable to spray drying. It may be remarked that apart from smaller numbers the distribution of coliform types in freshly dried milk differed considerably from that found in raw milk and very markedly from that in holder-pasteurized milk.

Previous work (30) has shown that coliform organisms died out fairly rapidly in dried milk during storage, whereas anaerobic spores survived for long periods. Consequently false positive tests became even more frequent and comprised 79% of all positive tests obtained from stored powder samples. Of the true positives, however, the *coli* I types died out most rapidly whilst the *cloacae* types survived the longest. These findings accord with the general belief that the *aerogenes-cloacae* group is the most resistant to drying under natural conditions, e.g. in dust and on vegetation.

Sweetened condensed milk

Sweetened condensed milk is not a sterile product, but depends upon the high sucrose content for its preservation. Normally the number of coliform organisms present is very small, and inoculations of at least 10 ml. of reconstituted milk are required for their detection. (Throughout this work reconstituted milk was prepared by dissolving 10 g. condensed milk in 90 ml. sterile water at 50° C.) Moreover, as in the case of dried milk, coliform organisms die out in condensed milk during storage.

Nevertheless, coliform organisms were formerly of fairly common occurrence owing to the notoriously poor hygienic standards prevalent in condenseries. A comprehensive review of bacteriological investigations prior to 1932 has been given by Allen (46), and although little detailed information concerning coliform organisms is available it would appear that earlier investigators frequently found these organisms in condensed milk. Nevertheless, although coliform organisms were able to survive the high sucrose concentration they were unable to grow, and gassy fermentations of condensed milk were almost invariably traced to the action of yeasts. The only recorded instances of gas production by coliform organisms appear to have been associated with under-concentration of the condensed milk.

Hygienic standards have, however, been gradually improved, and during recent years this improvement has been quite marked. Thus of thirty-seven samples of various makes purchased at random in the open market during 1938–40, 43·2% yielded presumptive positive coliform tests (Table 8); fifty-five similar samples obtained during 1943 yielded only 7·3% presumptive positives. Some of the factors involved have been elucidated by comparing data obtained at an old plant (not now in operation) and a very modern plant.

Table 8. *Sweetened condensed milk*
Incidence of presumptive positive tests

Old plant		New Plant	
	Presumptive positives		Presumptive positives

Table 8. *Sweetened condensed milk*

Incidence of presumptive positive tests

Old plant	Total tests	Presumptive positives No.	Presumptive positives %
Pasteurized milk (holder process)	45	9	20·0
Sugar	20	Nil	—
Condensed milk from vacuum pan	57	44	77·2
Condensed milk from cooler	76	68	89·5
Condensed milk in sealed cans, tested when 1 day–4 weeks old	90	50	55·5
Miscellaneous cans of other makes, including imported produce, examined during 1938–40, age at testing unknown	37	16	43·2
Miscellaneous cans of other makes, all British, examined during 1943; age at testing unknown but over 12 months	55	4	7·3

New Plant	Total tests	Presumptive positives No.	Presumptive positives %
Heated milk from forewarmer and hot well	38	Nil	—
Swabs of forewarmer	8	Nil	—
Condensed milk from vacuum pan	33	1	3·0
Swabs of condensing plant	6	Nil	—
Condensed milk from drop and weighing tanks	19	1	5·3
Swabs of drop and weighing tanks	66	8	12·1
Condensed milk from coolers	21	3	14·3
Condensed milk from storage tanks	6	Nil	—
Swabs of storage tanks	29	Nil	—
Condensed milk from bowl or nozzle of can filler	12	10	83·3
Swabs of filler bowl and nozzle	6	1	16·7
Swabs of can lid conveyor	17	10	58·8
Rinses of wiping cloths	2	1	50·0
Rinses of empty cans and lids	24	Nil	—
Condensed milk in sealed cans:			
Season (1) Examined when 1–6 weeks old	118	Nil	—
Season (1) Examined when 12 months old	23	2	8·7
Season (1) Examined when 18 months old	79	1	1·3
Season (2) Examined when 1–30 hr. old	182	60	33·0
Season (2) Examined when 2–5 months old	92	4	4·3

Incidence of coliform types

	No. presumptive positives examined	False positives: Yeasts	False positives: Anaerobes	No. true positives containing mixed strains	Total coliform cultures	*Coli* I	*Coli* II	Intermediate I	Intermediate II	*Aerogenes* I	*Aerogenes* II	*Cloacae*	Irregular III	Irregular V	Irregular VIII
Old plant															
Holder-pasteurized milk	9	—	—	1	10	8	—	—	—	2	—	—	—	—	—
Condensed milk, vacuum pan	44	—	—	14	58	14	—	—	—	40	—	2	—	—	2
Condensed milk, cooler	59	4	8	15	62	16	—	3	—	36	—	5	—	—	2
Condensed milk, sealed cans	50	4	6	8	48	12	—	—	—	34	—	2	—	—	—
Total	162	8	14	38	178	50	—	3	—	112	—	9	—	—	4
		13·6%		27·1%		28·1%		1·7%		68·0%			2·2%		
Modern plant															
Condensed milk, can filler	3	—	—	—	3	3	—	—	—	—	—	—	—	—	—
Condensed milk, sealed cans	44	—	4	1	41	14	2	1	2	17	4	—	—	—	1
Total	47	—	4	1	44	17	2	1	2	17	4	—	—	—	1
		8·5%		2·3%		43·2%		6·8%		47·7%			2·3%		
Total, both plants	209				222	67	2	4	2	129	4	9	—	—	5
						31·1%		2·7%		64·0%			2·2%		

Prior to the concentration process at the old plant, the milk was subjected to normal holder pasteurization at 145° F. for 30 min., but was not subjected to a cooling process. Some valve leakages occurred from holder compartments, and examination of 10 ml. quantities of the pasteurized milk yielded 20% of coliform positives, all of which were true positives (Table 8). Of ten coliform cultures isolated from this source eight were *coli* I and two *aerogenes* I strains.

The pasteurized milk (which gradually cooled to about 120° F.) was mixed with the sugar in a mixing tank, from which the sweetened milk was drawn into the vacuum pans for concentration. The sugar was added as a hot syrup which was always found free from coliform organisms.

Samples drawn from the vacuum pans at various stages throughout the condensing process commonly contained coliform organisms, and 77·2% of all such samples yielded positive coliform tests. The additional coliform contamination was derived from the coil-type vacuum pans. Certain portions of this plant, notably surfaces behind the coils and in the vapour pipe, were extremely difficult of access for cleaning purposes and tended to accumulate deposits; moreover, the extremely large size of the plant rendered effective steam sterilization virtually impossible. Quantitative estimations of coliforms present in samples drawn from the vacuum pans at intervals throughout the condensing process showed a definite decline in numbers as the degree of concentration increased, due no doubt to the effects of increasing osmotic pressure. (The temperature during concentration under vacuum was about 130° F.) Moreover, the coliform flora of vacuum-pan samples (Table 8) contained a high proportion of *aerogenes-cloacae* types, and detailed inspection of records showed that the predominance of these types became more marked as the concentration increased. From scrapings of vacuum-pan deposits five coliform cultures were isolated of which three were *aerogenes* and two *cloacae* strains.

The condensed milk was cooled in vats fitted with a horizontal rotating cooling coil, and some additional coliform contamination at this stage was probably derived from bearing glands in contact with the condensed milk.

In general, therefore, the conditions appeared to favour a selective survival of *aerogenes-cloacae* strains; these probably originated partly as survivors of low-temperature pasteurization of the original milk and partly from contaminated surfaces of condensed milk plant, whilst the sugar was not a source of coliform organisms.

Apart from particular items of plant, the operating conditions at the modern plant differed in some important respects. The milk was pre-heated to 172–175° F. by passage through tubular heaters and was subsequently held at this temperature for 15 min. in a forewarmer wherein the sugar was added to the milk. The sweetened milk was then drawn into the vacuum pan and was invariably free from coliform organisms (Table 8); consequently any coliform organisms found in vacuum-pan samples must have been derived from vacuum-pan surfaces. Two types of vacuum pan were in use, viz. a coil type similar in general design to those employed at the old plant, but of modern construction, and a forced circulation calandria type. The calandria type was more readily cleaned and consistently produced condensed milk containing fewer bacteria than did the coil-type pan, but for all practical purposes the condensed milk discharged from either pan was free from coliform organisms.

The condensed milk discharged from the vacuum pan into a drop tank and thence to a weighing tank, from which it was pumped through a continuous-flow tubular cooler into

large bulk-storage tanks. All the foregoing items of plant and the connecting pipelines were subjected to both steam and hypochlorite sterilization.

Condensed milk samples taken from the drop tank, weighing tank, coolers, and bulk-storage tanks were usually free from coliform organisms. In view of the small numbers of coliforms involved, further information was sought by swabbing plant surfaces after the cleaning and sterilizing processes had been completed. (Cooler surfaces could not be swabbed.) On a few occasions very small numbers of coliform organisms were present in such swabs of the drop and weighing tanks, but none were swabbed from bulk-storage tanks (Table 8). Most of the coliform-positive swabs were obtained from tank outlet cocks; great care was needed to ensure satisfactory cleaning of condensed milk cocks and pipelines owing to the ready accumulation of sticky condensed milk deposits in such situations. In general, however, the cleaning and sterilizing processes were satisfactory and, in contrast to findings at the old plant, the condensed milk prior to filling into cans was free from coliform organisms.

Can-filling and sealing operations were undoubtedly the major sources of coliform organisms. The cans and lids themselves before use were always free from coliforms, but swabs of filling and lid-seaming machinery revealed serious sources of contamination which necessitated the introduction of special precautionary measures. The difficulties were increased by the sticky nature of the condensed milk itself and by the construction of the machines which rendered very difficult either steam or hypochlorite sterilization of some parts which contacted the milk. Some temporary stoppages for adjustment of machines involved manual contact with sticky parts, and great care was needed to avoid cumulative contamination of both machine surfaces and operatives' hands. Moreover, it was necessary, following such stoppages, to wipe residues from sticky surfaces, and the cloths employed became seriously contaminated until steps were taken to maintain a supply of sterilized cloths. The turret-head plate (a slide which conveyed the lids to the point of seaming on to the filled cans) was particularly difficult to maintain free from spillage or drippings of condensed milk, and swabs frequently yielded coliform positives (Table 8). Finally, bacterial proliferation would almost inevitably occur in any residues of diluted condensed milk left behind on the machines after washing.

Consequently condensed milks sampled from the can filler, or from sealed cans immediately after filling, yielded many coliform-positive tests, but canned condensed milks examined after a period of storage (even if only a few weeks) yielded very few positives. Thus at the old plant, samples tested after canning (Table 8) showed a reduced incidence of coliform positives, whilst at the new plant during season 1 only three positives were obtained from 220 samples. It was suspected that these results were related to the age at testing, and during season 2 at the new plant a series of 182 samples was examined within a few hours of canning. Coliform-positive tests were obtained from 33% of these samples. Many of the samples were canned during hot weather, and detailed records revealed a close correlation between the occurrence of coliform positives and high atmospheric temperature; this correlation supported the probability of bacterial multiplication on the surfaces of canning machinery. It is hoped, however, to present detailed data in a later paper. Nevertheless, ninety-two samples of the same series yielded only 4·3% of coliform positives when tested after 2–5 months' storage. It was therefore clear that coliform organisms not only failed to multiply in condensed milk, but declined in numbers during

storage. This fact, if generally true, must seriously limit the utility of the coliform test in the examination of condensed milk.

Examination of the coliform types isolated from the new plant (Table 8) revealed a much higher proportion of *coli* strains (43·2%) than was found at the old plant, but the *aerogenes-cloacae* strains still comprised 47·7% of the cultures. Intermediate strains were probably slightly more numerous at the new plant. A further indication of different conditions prevailing at the two plants was provided by extreme contrasts in the occurrence of mixed strains, viz. 27·1% at the old plant and 2·3% at the new plant.

Of the total of 222 cultures isolated from both plants, 64·0% were placed in the *aerogenes-cloacae* group, 31·1% in the *coli* group, and 4·9% in the intermediate-irregular group. It would appear that *aerogenes-cloacae* strains are probably the typical coliforms of sweetened condensed milk. In this connexion it may be noted that James(45) isolated a sugar-tolerant *cloacae* strain able to grow anaerobically in 30% solutions of cane sugar. The *aerogenes* I type comprised the majority of the *aerogenes-cloacae* cultures, and the *coli* I type greatly outnumbered the *coli* II type. All the five irregular cultures obtained were grouped as type VIII.

Bulk cream

Very large quantities of liquid cream are distributed in bulk from country creameries to smaller retailers who repack it without further treatment into small containers for domestic use. Bulk cream is usually distributed in metal cans ranging from 1 qt. to 2 gal. capacity although considerable quantities are also transported in churns (10–17 gal.) to the larger distributors. Since allowance must be made for transport in bulk to the retail distributor, for retail distribution, and for a short period of domestic storage, a long-keeping quality is essential. Consequently the cream invariably receives some form of heat treatment at the producing creamery and more than one heat treatment is not uncommon.

The type of heat treatment varies between different creameries and both H.T.S.T. and holder methods are employed, although the former is more common. The method of processing may considerably influence properties such as flavour and viscosity, but in any case the heat treatment is more severe than that employed in the pasteurization of milk, and the number of organisms present in freshly heated cream is therefore small. General bacteriological data relating to bulk-cream manufacture will, it is hoped, be presented at some future date, but some relevant conclusions may be stated here. The total colony counts of processed bulk-cream samples are usually very low, commonly a few hundred per ml., and bear no close relationship to the actual keeping qualities of the samples. The presence of coliform organisms in the flora, even if the total of all organisms is small, does, however, markedly reduce the keeping quality. The elimination of coliform contamination is therefore of considerable practical importance.

Summarized data relating to the coliform flora of processed bulk cream are given in Table 9. H.T.S.T. processing (at 180° F. for 20 sec.) and cooling (to 35–40° F.) was carried out by means of a plate-type heater and cooler. Cream samples taken directly from the discharge of this plant yielded 6·1% of presumptive positives. The presence of coliform organisms at this point was found to be due, on different occasions, to inadequate preliminary sterilization of the plant, or to underheating of the cream at the beginning of a run (resulting in recontamination of the cooling section), or to infection of worn rubber gaskets between the plates. Cream processed by the H.T.S.T. method, but cooled by

passage over an open capillary-type cooler, yielded the much higher proportion of 19·7% presumptive positives.

The processed cream was distributed by means of a filling machine into bulk transport cans or, in the case of larger quantities, directly into churns. In either case the containers of cream were held in cold storage for at least 24 hr. before dispatch, after which period all samples were taken.

Table 9. *Pasteurized bulk cream*

Incidence of presumptive positive tests

	Total tests	Presumptive positive tests	
		No.	%
H.T.S.T. process (180° F., 20 sec.):			
Direct from plate-type heater and cooler	426	26	6·1
H.T.S.T. cream cooled by open capillary-type cooler	335	66	19·7
Cream from churns after 24 hr. storage	793	218	27·5
Cream from filling machine	475	169	35·6
Cream from bulk cans after 24 hr. storage	258	45	17·4
Holder process (145° F., 60 min.):			
Cream after heating	48	Nil	Nil
Cream after cooling and filling into bulk cans, 24 hr. storage	73	14	19·2
Cream pasteurized in sealed containers	42	1	2·4
Cream after handling by distributors, various sources and processes	70	24	65·7

Incidence of coliform types

	H.T.S.T. process				Holder process			
	Direct from plate heater and cooler	H.T.S.T. cream cooled by open capillary cooler	Filling machine	Churns or bulk cans	After heating and cooling	Cream from distributors (various processes)	Total	
No. presumptive positives examined	20	8	49	110	12	15	214	
False positives (anaerobes)	2	Nil	10	14	6	Nil	32 (14·9%)	
No. true positives containing mixed strains	4	Nil	3	11	2	3	23 (12·6%)	
Total coliform cultures	22	8	42	107	8	18	205	
Coli I	12	2	12	46	6	7	85	46·3%
Coli II	—	—	2	6	2	—	10	
Intermediate I	—	—	4	7	—	—	11	6·3%
Intermediate II	—	—	—	2	—	—	2	
Aerogenes I	6	—	12	35	—	7	60	36·6%
Aerogenes II	—	—	2	9	—	—	11	
Cloacae	—	4	—	—	—	—	4	
Irregular III	2	—	8	—	—	—	10	10·7%
Irregular V	—	—	2	2	—	4	8	
Irregular VIII	2	2	—	—	—	—	4	

Post-heating processes were undoubtedly the major cause of coliform contamination; thus 793 samples from churns yielded 27·5% presumptive positives, 475 samples from the filling machine yielded 35·6% presumptive positives, and 258 samples from bulk cans yielded 17·4% of presumptive positives. As in the case of pasteurized milk, the coliform test revealed slight plant contamination which was not detected by the total colony count.

Cream subjected to a holder process at 145° F. for 60 min. was invariably free from coliforms when leaving the holder, but samples taken after cooling, filling into bulk cans, and cold storage for 24 hr. yielded 19·2% of presumptive positives—almost the same proportion as was obtained from similar samples treated by the H.T.S.T. process.

Quantitative estimations revealed presumptive positives in minimum quantities varying from 10 to 0·01 ml. of cream, but only a small proportion of the samples gave positive reactions from less than 1 ml. of cream. Moreover, a considerable number of reactions were false positives (50% in the case of holder-process samples). Nevertheless, considerable proliferation might occur during distribution in hot weather, and it seemed clear that coliform contamination could be almost avoided by eliminating all post-heating handling of the cream. With this object in view a small-scale process was introduced which employed heating (holder method) and cooling of the cream in the sealed bulk container in which it was to be dispatched. Only one presumptive positive (actually a false positive) was obtained from forty-two samples of cream processed in the container. Whilst some practical difficulties arose, these were not insuperable except in the case of very large containers such as churns.

Opportunity was taken to sample pasteurized cream distributed from a number of other sources, and 65·7% of presumptive positives were obtained from seventy such samples.

Confirmatory tests of 214 presumptive positives revealed an incidence of 14·9% false positives, and 12·6% of the true positives contained mixed coliform strains. The distribution of coliform strains was quite different from that found in either raw or pasteurized milk. The *coli* types comprised the largest group (46·3%) of cultures, but these organisms were much less abundant than in holder-pasteurized milk and less frequent than in raw or H.T.S.T.-pasteurized milk. Within the *coli* group the *coli* II type was relatively common. The *aerogenes-cloacae* group (36·6% of cultures) was nearly as numerous as the *coli* group; the predominant strain was *aerogenes* I, but type II strains were relatively more frequent than in other products previously discussed. Of the remaining cultures, an interesting feature was the comparative frequency (10·7%) of irregular strains.

There was some indication of more generalized distribution of all coliform strains rather than a complete predominance of *coli* I and *aerogenes* I strains. This might be expected from the somewhat variable nature of the coliform contamination arising from the various handling and packing operations. The majority of cultures were isolated from H.T.S.T.-processed cream, and no special circumstances were evident such as large items of plant maintained at high temperatures, as in the holder pasteurization of milk. Heat-resistance tests (Table 15) yielded 26·9% of resistant cultures, a lower proportion than was observed among cultures isolated in connexion with the pasteurization of milk or the manufacture of milk powder.

Pont(46), in Australia, isolated coliforms from 72% of 112 samples of raw cream. The distribution of coliform types was very different from that observed in the present study of pasteurized cream (viz. 76·5% *coli*, 11·0% *aerogenes-cloacae*, and 12·5% intermediate). Pont reported that *aerogenes* and intermediate cultures produced more rapid and serious deterioration than *coli* cultures when inoculated into fresh raw cream of good quality. Although the bacteriological methods employed were very similar to those described in this paper the samples were not comparable, since they were taken from raw cream delivered in bulk to butter factories and which had not been subjected to the packing processes inseparable from the distribution of liquid cream.

CLOTTED CREAM

The methods of manufacture and the bacteriological flora of clotted cream have been described in a previous paper (47), but no other references to this product have been found in the literature.

Data relating to the coliform flora are summarized in Table 10. When the method of manufacture employed the scalding of a layer of cream floating on the surface of milk, the original milk and cream mixture as delivered into the scalding trays sometimes contained

Table 10. *Clotted cream*

Incidence of presumptive positive tests

	Total tests	Presumptive positive tests	
		No.	%
Scalding on milk:			
(*a*) Milk and cream mixture after preliminary pasteurization	92	14	15·2
(*b*) Cream layer immediately after scalding	68	Nil	Nil
(*c*) Clotted cream after cooling, skimmed aseptically into sterile sample bottles	53	Nil	Nil
(*d*) Clotted cream after cooling, skimmed by factory methods into bulk storage containers	172	50	29·1
Direct scalding in trays, before removal to bulk storage containers	15	Nil	Nil
Packed clotted cream:			
(*a*) Scald on milk, water cooling	520	227	43·6
(*b*) Scald on milk, air cooling	83	33	39·8
(*c*) Direct scald in trays	132	27	20·5
Clotted cream as received by retailer (summarized figures for 8 factories)*	858	390	45·4*

Incidence of coliform types

	Pasteurized milk and cream	Clotted cream, skimmed into bulk-storage containers	Clotted cream, packed for dispatch	Total, all processes	
No. presumptive positives examined	9	49	62	120	
False positives: Yeasts	Nil	Nil	1	1	24·2%
Anaerobes	Nil	13	15	28	
No. true positives containing mixed strains	2	3	2	7 (7·7%)	
Total coliform cultures	11	39	48	98	
Coli I	6	16	16	38	47·9%
Coli II	2	1	6	9	
Intermediate I	—	1	2	3	3·1%
Intermediate II	—	—	—	Nil	
Aerogenes I	2	16	7	25	38·8%
Aerogenes II	—	—	6	6	
Cloacae	—	3	4	7	
Irregular III	—	—	—	Nil	10·2%
Irregular IV	—	—	1	1	
Irregular V	1	2	4	7	
Irregular VIII	—	—	2	2	

* Varied from 39 to 100%.

coliform organisms. This mixture was subjected to preliminary 'holder' pasteurization, and the coliform contamination normally associated with this process was liable to arise. Ninety-two such samples yielded 15·2% presumptive positives from 10 ml. inoculations; eight of eleven coliform cultures isolated were *coli* strains.

The subsequent scalding process at 180–190° F. destroyed all coliforms, and even at the conclusion of the long cooling process aseptically skimmed samples of the clotted

cream were free from coliforms although proliferation of other bacteria was evident; it was concluded that coliform infection from the atmosphere did not occur.

Similarly, when clotted cream was prepared by direct scalding of thick cream (60% fat content) in shallow trays, no coliform organisms were found after the cream had been scalded and cooled.

Unfortunately, the product is unsuited to mechanical handling, and the clotted cream must, in the first method, be skimmed from the surface of the milk layer, or in the second removed from the scalding trays by hand methods employing special utensils. The cream thus removed was normally transferred to bulk-storage containers and placed in cold storage prior to packing by hand into cans of 1–20 lb. capacity, in which it was dispatched to retail distributors. Under such circumstances it was difficult to avoid periodic contamination from utensils or by manual contact.

The figures quoted in Table 10 reveal the significance of these sources of contamination. Thus the operation of skimming the cream from the milk layer was followed by the occurrence of 29·1% presumptive positives from a series of 172 samples. The further operations incidental to packing for dispatch also contributed a further quota, and three series prepared by different methods but sampled after packing into final transport containers yielded 43·6, 39·8 and 20·5% of presumptive positives. The general bacteriological quality of clotted cream was inferior to that of pasteurized liquid cream, and the superior value as a control method of the coliform test compared with the total colony count was therefore less pronounced in the former product.

Clotted cream is consumed mainly during hot weather and considerable bacterial multiplication is unavoidable during distribution. A series of 858 samples taken from bulk containers as received by retail distributors from eight factories yielded 45·4% presumptive positives, varying from 39 to 100% for the individual factories. Among these samples the presence of coliforms in 0·001 ml. of cream was not uncommon. No account was taken of further handling during retail distribution, but additional contamination from this source seemed highly probable.

In view of the fact that souring is the commonest defect of clotted cream, and of the high atmospheric temperatures to which it is frequently exposed, it seems likely that coliform organisms play a large part in the deterioration of this product.

Detailed tests of ninety-eight cultures gave a distribution of 47·9% *coli*, 38·8% *aerogenes-cloacae*, 10·2% irregular and 3·1% intermediate types. It seemed clear, therefore, that the various skimming and packing operations resulted in the occurrence of a large proportion of non-*coli* strains, and the majority of the irregular cultures were isolated from packed samples.

It was of interest that the incidence of the main coliform groups was almost identical with that found in the manufacture of pasteurized liquid cream, and the relative frequency of *coli* II and *aerogenes* II strains was even more noticeable than in the liquid product. Such findings were no doubt due to the similarity in general chemical composition of the two products and to the fact that in both manufacturing processes the major contamination was derived from packing operations.

It may also be noted that *cloacae* strains were not infrequent in clotted cream, whilst the only culture of irregular IV to be isolated during this work was obtained from packed clotted cream.

BUTTER

The coliform flora of defective butter has been studied by previous investigators, and it is generally accepted that organisms of the *aerogenes-cloacae* group are important agents in the production of various off-flavours, particularly in unsalted butter. Thus Hammer & Yale (48) identified twenty-five coliform cultures isolated from off-flavoured butter as eighteen *aerogenes*, four *cloacae*, and three intermediate strains; no *coli* strains were found. These authors also prepared experimental butters from cream inoculated with various

Table 11. *Butter*

Incidence of presumptive positive tests

	Total tests	Presumptive positives No.	Presumptive positives %	
Cream direct from pasteurizer and cooler	40	2	5·0	
Cream from storage vessel	44	30	68·2	
Cream from butter churn immediately before churning	30	22	73·3	
Butter grain after washing	36	30	83·3	
Butter after salting	36	22	61·1	
Butter from boxes, various periods of cold storage	74	37	50·0	57·0
English butter from other creameries, as purchased	52	40	76·9	
Whey butter churned from unpasteurized cream	26	26	100·0	
Imported butter, unsalted (i.e. under 2·0% salt), as purchased	62	36	58·1	
Imported butter, salted	70	11	15·7	
Blended and packeted butter	168	125	74·4	

Incidence of coliform types

	Cream in storage vessel	Cream in butter churn	Butter grain washed	Butter after salting	English butter in cold storage	Imported butter: Unsalted	Imported butter: Salted	Blended butter	Total	
No. presumptive positives examined	30	8	16	30	78	26	8	71	267	
False positives: Yeasts	Nil	Nil	Nil	Nil	4	Nil	Nil	3	7	7·1%
Anaerobes	2	Nil	Nil	Nil	4	Nil	2	4	12	
No. true positives containing mixed strains	8	2	6	8	12	8	4	11	59 (23·8%)	
Total coliform cultures	38	10	22	38	83	34	10	75	310	
Coli I	16	2	10	14	38	20	4	24	128	46·4%
Coli II	4	2	Nil	Nil	2	2	Nil	6	16	
Intermediate I	4	4	2	4	Nil	Nil	Nil	10	24	8·4%
Intermediate II	Nil	Nil	Nil	2	Nil	Nil	Nil	Nil	2	
Aerogenes I	6	2	8	10	40	4	4	25	99	43·9%
Aerogenes II	6	Nil	2	4	2	Nil	Nil	2	16	
Cloacae	2	Nil	Nil	4	1	6	2	6	21	
Irregular III	Nil	Nil	Nil	Nil	Nil	2	Nil	Nil	2	1·3%
Irregular V	Nil	Nil	Nil	Nil	Nil	Nil	Nil	2	2	
Irregular VIII	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	

pure coliform cultures; 3–20% of the coliform organisms present in the cream were retained in the butter. Storage experiments showed that *aerogenes-cloacae* cultures grew more readily in butter and were more salt tolerant than *coli* cultures, whilst the *aerogenes-cloacae* cultures regularly produced off-flavours and odours, but the *coli* cultures rarely did so. Grimes (16) found that low-temperature *aerogenes-cloacae* strains having an optimum growth temperature around 25° C. were also of importance.

The object of the present investigation was to study the general coliform flora associated with the manufacture and storage of normal butter of satisfactory quality. A summary of the recorded results is given in Table 11.

The pasteurized cooled cream when sampled directly from the pasteurizer (plate type) was free from coliform organisms; forty samples yielded only two presumptive positives, both of which were false positives. Cream held for ripening in storage vessels, however, showed 68·2% presumptive positive tests, whilst cream placed in the butter churns and sampled just before churning commenced yielded 73·3% of positive tests. This considerable post-pasteurization infection was attributed to inadequate steam sterilization of storage vessels, whilst wooden butter churns could not be steamed at all. Recent work on the use of supplementary hypochlorite sterilization has resulted in marked improvement, and swabs of both storage vessels and wooden churns have given uniformly negative coliform tests. Samples of the washed butter grain showed 83·3% positive tests, but the same butters after salting gave the reduced figure of 61·1% positives. Quantitative estimations also showed that even in the positive samples the number of coliforms was reduced by salting, so that some of the organisms were undoubtedly very susceptible to the presence of salt. (All the butters contained less than 2% salt.) Further samples of English butter packed in boxes were examined after varying periods of cold storage. The samples manufactured in the creamery from which other plant samples had been examined yielded 50·0% of presumptive positive tests, so that a slight decline in the incidence of coliforms may possibly have occurred during storage. Samples of English butter purchased from other creameries, however, gave the higher figure of 76·9% positive tests. Since all the samples of stored English butter gave a mean figure of 57% positive tests, it was concluded that coliform organisms were of common general occurrence in the butter flora. The prevention of coliform contamination was rendered difficult by the very extensive use of wooden equipment which could not be sterilized by steam, and by some inevitable manual contact. On the other hand, this work also showed that it was practicable to maintain freedom from coliform contamination throughout the whole butter-making process.

It may be noted that no false positive tests were obtained from samples taken during the manufacturing process, but a few such tests, some of which were due to yeasts, were recorded from samples of stored butter.

A considerable proportion of the butter consumed in Britain is imported from overseas, particularly from Australia and New Zealand, and the coliform flora of such butter following many months' holding and transport in cold storage was thought to be of interest. Moreover, much butter as retailed consists of 'blended butter' sold ready wrapped in ½ or 1 lb. blocks. Various imported butters, and possibly English butter, are blended together by machinery to produce standard brands, which are packeted by automatic machinery; this process is often carried out in factories which also manufacture English butter. During blending and wrapping operations further coliform infection may result from manual contact or contamination from machines.

A series of imported butters (Table 11) as used for blending purposes was examined. All samples were taken from butter graded as first quality in the country of origin; none of the butters was less than 6 months old and some were probably much older. Samples of unsalted butter gave 58·1% of presumptive positive tests, whilst salted butters gave only 15·7% of positive tests. Rice(49), in Australia, obtained 46·6% of positive tests from 131 samples of freshly made Australian salted butter. He also observed a slow decline in the numbers of coliforms when the butters were held in cold storage at 14° F., whilst little increase in numbers was found in salted butters held at 60° F., but pronounced increases occurred in unsalted butters held at 60° F.

It may therefore be assumed that during prolonged cold storage a slow decline of the coliform population occurs, the extent depending upon the degree of salting. The data herein recorded for both English and imported butter support this conclusion.

A further series of blended, packeted butters yielded 74·4% of positive tests from 168 samples. Since this figure was higher than those recorded for either English or imported original butters, the additional handling involved in the preparation of wrapped blended butter must have contributed further coliform organisms.

A total of 310 coliform cultures isolated from all sources was grouped as 46·4% *coli*, 43·9% *aerogenes-cloacae*, 8·4% intermediate, and 1·3% irregular types. The *coli* and *aerogenes-cloacae* groups, in almost equal numbers, therefore comprised the bulk of the general coliform flora, but examination of the individual series revealed interesting variations.

Cultures obtained from cream in storage vessels gave 52·7% *coli*, 36·8% *aerogenes-cloacae*, and 10·5% intermediate-irregular strains. These figures are fairly similar to those obtained for pasteurized bulk cream (Table 9). Of ten cultures isolated from cream in the butter churn, four were *coli*, four intermediate, and two *aerogenes* types. Sixteen cultures were isolated from swabs of butter churns prepared for reception of the cream (Table 2); of these five were *coli*, four intermediate, and seven *aerogenes-cloacae* types. The series of cultures obtained from the washed butter grain in the churn, after salting, and from the butter in cold storage, showed a gradually increasing proportion of *aerogenes-cloacae* types, whilst intermediate types appeared to be absent from the stored butter.

The cultures isolated from stored imported butter revealed a high proportion of *coli* types in unsalted butter and a high proportion of *aerogenes-cloacae* types in salted butter; few intermediate cultures were isolated. The distribution of coliform types in blended butter appeared to indicate approximately equal numbers of *coli* and *aerogenes-cloacae* types, but considerable variation might be expected in particular blends according to the flora of the constituent individual butters. The intermediate-irregular group showed a marked increase in numbers, and these organisms were probably derived from contamination during blending and packing processes.

It may be added that Rice (50) found fewer *aerogenes-cloacae* and more *coli* and intermediate strains in freshly made Australian salted butter (Table 2).

In very general terms it may be concluded that *coli* strains are derived from post-pasteurization infection of the cream and to a lesser extent from subsequent plant infection; such strains may survive for lengthy periods in butter, particularly if unsalted. Intermediate-irregular strains are probably somewhat fortuitous in occurrence and may not survive long periods of cold storage. The general conditions of butter manufacture appear to favour the *aerogenes-cloacae* group, members of which may gain access at any stage of the process and subsequently survive in the cold-stored butter; in addition, they are evidently the most salt tolerant of the coliform organisms.

Soft cheese

The occurrence of coliform organisms at various stages during the large-scale manufacture of soft cheese was studied in connexion with two varieties of cheese, viz. (*a*) a low-acid, rather slow-draining type, and (*b*) a high-acid type, in which the milk was coagulated and drained more rapidly and at a higher temperature than in (*a*).

In both cases the raw milk was pre-heated before use and the pre-heated milk was

invariably free from coliforms (Table 12). Samples of curd taken at the time of removal from the bulk vats to draining vats contained coliform organisms quite frequently. During the early stages of draining (up to 4 hr.) the incidence of coliforms in the curd appeared to increase, but coliforms were found less frequently in samples of curd drained for 24 hr. Possibly during the early stages, when much whey was present in contact with the curd, rapid multiplication of coliform organisms occurred which more than balanced the loss of organisms removed in the whey. As draining progressed, less whey was present, and, in addition, the increasing acidity probably checked the rate of multiplication so that the effect of the removal of organisms in the whey became predominant. The amount of whey removed during pressing of the curd was much less than that lost during draining (only light pressing was employed), and samples of pressed curd yielded a higher proportion of positive coliform tests. The finished cheese was moulded into portions and wrapped in tinfoil by automatic machines; there was no evidence of additional coliform contamination following these processes. Positive coliform tests were obtained from 51·1% of samples of wrapped low-acid cheese and from 26·4% of wrapped high-acid cheese samples.

The coliform organisms were probably derived from the factory bulk-starter cultures, from the large vats and other equipment, from draining cloths, and from manual contact.

In comparison with the manufacture of hard cheese, soft-cheese making on a large scale is somewhat more 'mechanized', and the amount of manual contact involved is small. The draining cloths employed in soft-cheese manufacture were, however, a source of considerable bacteriological contamination. Coliform organisms were found commonly on these cloths and were difficult to eliminate. Thus cloths submitted to the normal process of boiling in a laundry wash boiler yielded 87·5% of coliform positives, although the water was actually raised to boiling-point; this was attributed to very slow heat penetration through a dense mass of material. The proportion of positive tests was reduced to 23·6% by means of additional steam treatment, but coliforms could only be eliminated by employing steam sterilization under pressure.

Fluctuations in the occurrence of coliforms throughout the manufacturing processes followed a similar course in both types of soft cheese, but the actual incidence of positive tests was much higher in the low-acid type. This is probably due to the much more frequent occurrence of coliforms in the bulk starter used for the low-acid cheese and to the longer draining period in contact with draining cloths.

Of the 398 presumptive positives examined (Table 12) only 8·0% were false positives and nearly half of these were due to yeasts. (The soft-cheese flora included yeasts of many types.) The true positives contained only 6·0% of mixed coliform cultures. The total collection of 393 cultures comprised 60·3% *coli*, 23·4% *aerogenes*, 8·4% irregular, and 7·9% intermediate cultures. The proportion of *coli* strains was therefore higher than in other manufactured products, whilst that of *aerogenes-cloacae* strains was correspondingly lower. The irregular types were also comparatively frequent in soft cheese.

Comparison of the separate data for the two types of soft cheese revealed a higher proportion of *coli* and a lower proportion of *aerogenes-cloacae* cultures from the low-acid type; it was also found that the coliform types present in the low-acid soft cheese were very similar to those observed in Cheddar cheese (Table 13).

Although none of the coliforms present in soft cheese appeared to be derived from the original pre-heated milk, the proportion of heat-resistant strains (46·2%) seemed to be higher than in pasteurized milk or any other manufactured product (Table 15). In view

Table 12. *Soft cheese*

Table 12. *Soft cheese*

Incidence of presumptive positive tests

	Low-acid cheese			High-acid cheese		
		Presumptive positives			Presumptive positives	
	Total tests	No.	%	Total tests	No.	%
Heated milk	68	Nil	Nil	80	Nil	Nil
Factory bulk starter	175	64	36·6	196	8	4·1
Curd when removed from bulk vats	76	48	63·2	80	16	20·0
Curd during first 4 hr. draining	36	30	83·3	22	14	63·6
Curd after 24 hr. draining	96	35	36·5	118	26	22·0
Curd after pressing	626	328	52·4	445	164	36·9
Wrapped cheese	178	91	51·1	159	42	26·4
Draining cloths, boiled	16	14	87·5	—	—	—
Draining cloths, steamed	178	42	23·6	—	—	—
Draining cloths, steamed under pressure	16	Nil	Nil	—	—	—

Incidence of coliform types

	No. presumptive positives examined	False positives		No. true positives containing mixed strains	Total coliform cultures	*Coli*		Intermediate		*Aerogenes*		*Cloacae*	Irregular		
Low-acid cheese		Yeasts	Anaerobes			I	II	I	II	I	II		III	V	VIII
Factory bulk starter	17	—	4	1	14	7	2	1	—	3	—	—	—	—	1
Curd when removed from bulk vats	12	—	—	1	13	11	—	1	—	1	—	—	—	—	—
Drained curd	14	—	—	2	16	12	—	—	—	2	—	—	—	—	2
Pressed curd	56	—	—	4	60	40	—	6	—	6	—	—	—	6	2
Wrapped cheese	50	8	—	Nil	42	26	—	4	—	8	2	—	—	—	2
Draining cloths	5	—	—	Nil	5	1	—	1	—	1	—	—	—	2	—
Total	154	8	4	8	150	97	2	13	Nil	21	2	Nil	Nil	8	7
		7·8%		5·6%		66·0%		8·7%		15·3%			10·0%		
High-acid cheese															
Curd when removed from bulk vats	20	—	2	Nil	18	14	—	—	—	4	—	—	—	—	—
Drained curd	23	—	—	2	25	21	1	—	—	3	—	—	—	—	—
Pressed curd	111	—	3	9	117	51	3	6	6	42	3	—	—	3	6
Wrapped cheese	90	6	9	3	83	42	6	6	—	9	3	5	—	—	9
Total	244	6	14	14	243	128	10	12	6	58	6	5	Nil	3	15
		8·2%		6·2%		56·8%		7·4%		28·4%			7·4%		
Total, all soft cheese	398	14	18	22	393	225	12	25	6	79	8	5	Nil	11	22
		8·0%		6·0%		60·3%		7·9%		23·4%			8·4%		

Table 13. *Cheddar cheese*

Incidence of presumptive positive tests

Normal cheese	Total samples	Presumptive positives No.	Presumptive positives %
Pasteurized milk, H.T.S.T. process	290	106	36·5
Starter, mother cultures	134	Nil	Nil
Starter, factory bulk cultures	256	90	35·1
Rennet	20	Nil	Nil
Cheddar curd, cutting to pitching	31	31	100·0
Cheddar curd, piling, milling, salting	33	33	100·0
Cheese, first 2 days	23	23	100·0
Cheese, 1st month of ripening	30	28	93·3
Cheese, 2nd month of ripening	57	45	78·9
Cheese, 3rd month of ripening	51	30	58·8
Cheese, over 3 months	70	14	20·0

Blown cheese	Total samples	Presumptive positives No.	Presumptive positives %
Rapid type:			
Pasteurized milk, H.T.S.T. process	14	14	100·0
Starter, factory bulk cultures	14	Nil	Nil
Cheddar curd, gassy	8	8	100·0
Blown cheese, 1–4 weeks	8	8	100·0
Slow type:			
Blown cheese, 2–3 months	44	43	97·7

Incidence of coliform types

Normal cheese	No. presumptive positives examined	False positives: Anaerobes	False positives: Yeasts	No true positives containing mixed strains	Total coliform cultures	*Coli*: I	*Coli*: II	Intermediate: I	Intermediate: II	*Aerogenes*: I	*Aerogenes*: II	*Cloacae*	Irregular: III	Irregular: V	Irregular: VIII
Pasteurized milk, H.T.S.T. process	20	—	—	10	34	10	6	1	—	13	1	2	1	—	—
Starter, factory bulk cultures	25	4	—	—	21	17	1	1	—	2	—	—	—	—	—
Cheddar curd, cutting to pitching	24	—	—	6	30	20	2	4	2	2	—	—	—	—	—
Cheddar curd, piling, milling, salting	24	—	—	18	58	16	3	19	—	9	—	—	2	3	6
Cheese, up to 2 days	17	—	1	3	20	12	3	2	—	2	1	—	—	—	—
Cheese, 1st month	27	—	—	9	36	27	2	2	—	5	—	—	—	—	—
Cheese, 2nd month	40	—	—	5	46	40	—	1	—	5	—	—	—	—	—
Cheese, 3rd month	25	2	—	—	23	22	1	—	—	—	—	—	—	—	—
Cheese, over 3 months	14	5	—	—	9	8	—	1	—	—	—	—	—	—	—
Total, all processes	216	11	1	51	277	172	18	31	2	38	2	2	3	3	6
		5·6%		32·7%		68·6%		11·9%		15·2%			4·3%		
Blown cheese															
Rapid type. Pasteurized milk, H.T.S.T.	10	—	—	9	24	6	5	2	—	10	1	—	—	—	—
Rapid type. Cheddar curd	6	—	—	6	14	4	—	4	—	6	—	—	—	—	—
Rapid type. Blown cheese, 1–4 weeks	8	—	—	—	8	8	—	—	—	—	—	—	—	—	—
Slow type. Blown cheese, 2 months	28	—	—	—	28	28	—	—	—	—	—	—	—	—	—
Slow type. Blown cheese, 3 months	16	1	—	—	15	15	—	—	—	—	—	—	—	—	—

Association of strains in rapid blowing cheese

Material	Strains found	No. positives	
Pasteurized milk	Mixture of *aerogenes* I, *coli* I, *coli* II	3	10 presumptive positives
	Mixture of *aerogenes* I, *coli* I	2	
	Mixture of *aerogenes* I, *aerogenes* II, *coli* II	1	
	Mixture of *aerogenes* I, *coli* II	1	
	Mixture of *aerogenes* I, intermediate I	1	
	Mixture of *aerogenes* I, *coli* I, intermediate I	1	
	Aerogenes I only	1	
Cheddar curd	Mixture of *aerogenes* I, *coli* I, intermediate I	2	6 presumptive positives
	Mixture of *aerogenes* I, *coli* I	2	

of the high proportion of *coli* cultures a correspondingly large number of heat-resistant strains might be anticipated, whilst in addition the draining cloths previously mentioned were probably a specially significant source of heat-resistant coliforms.

Finally, it may be observed that no relationship could be established between the presence of coliform organisms and any properties of the cheese, e.g. flavour, physical characteristics, or keeping quality.

CHEDDAR CHEESE

Some preliminary observations concerning the coliform flora of Cheddar cheese have been described in an earlier paper (50), and these results, together with some additional data, are shown in Table 13. The investigation was confined to factory cheese made from pasteurized milk.

(*a*) *Normal cheese*

The milk was pasteurized by a H.T.S.T. process, but no thermostatic control or diversion valve was employed. Post-pasteurization infection from coolers or pipelines sometimes occurred, and at one factory contamination of the bulk starter was fairly frequent. Coliform organisms were found in 36·5% of samples of pasteurized milk delivered to the cheese vats and in 35·1% of all samples of bulk factory starters. (In a number of cases the positive tests were obtained only from 10 ml. inoculations.) Mixed coliform cultures were obtained frequently from the pasteurized milk, and the flora consisted of *coli* and *aerogenes-cloacae* types in equal numbers. No starter sample, however, yielded mixed coliform types, and the majority of the positive tests were due to pure cultures of *coli* I strains; some false positives were obtained. Although the mother starter cultures were invariably free from coliform organisms, contamination occurred readily during preparation of the bulk starter unless stringent precautions were observed. No coliforms were isolated from rennet.

It was therefore possible to eliminate coliform contamination up to the stage of cutting the curd, provided that coolers, pipelines, and cheese vats were adequately sterilized and the bulk starter free from coliform infection. Throughout the later stages of cutting, pitching, piling and milling the curd, some coliform contamination invariably occurred, due no doubt to unavoidable manual handling and to contact with equipment such as wooden stirring rakes. The coliform flora commonly consisted of mixed types, and although *coli* strains were probably the most frequent, members of all the other coliform groups were isolated. The most noticeable feature was the common occurrence of intermediate I strains, particularly during the later stages of piling and milling the curd.

Many coliform strains, however, appeared to be short-lived, and the new cheese when removed from press to store already showed a decline of strains other than *coli* I. Nevertheless, cheese sampled up to 1 week old yielded 100% of positive coliform tests from heavy inoculations. As storage lengthened, however, fewer coliforms were found, and 93·3% of positive tests were obtained after 1 month, 78·9% after 2 months, 58·8% after 3 months and 20·0% over 3 months. The decline in numbers was accompanied by increasing predominance of *coli* I strains. This was confirmed by periodic examination of cheese originally containing coliform mixtures; after 4 months' storage *coli* I strains were the sole survivors.

Of the total collection of 277 cultures from all sources, 68·6% were *coli*, 15·2% *aerogenes-cloacae*, 11·9% intermediate and 4·3% irregular types. False positives (5·6%) were

infrequent and confined to either samples of bulk starters or cheese ripened for 3 months or more. The *coli* types were therefore isolated more frequently from cheese than any other source excepting holder-pasteurized milk. Heat-resistance experiments yielded 31·7% of resistant cultures (Table 15), a very similar figure to that obtained for pasteurized milk cultures.

It was concluded that although sporadic infection by varied coliform types may occur during the cheese-making process, the conditions prevailing throughout the making and ripening processes as a whole tend to favour the *coli* I strains, although the normal ripening process produces unfavourable conditions for all coliforms. *Coli* strains also predominated in soft cheese manufacture (p. 268).

(*b*) *Blown cheese*

It is generally recognized that coliform organisms are associated with the occurrence of blown cheese, and it is often stated that *aerogenes* strains are the major cause of this defect. Clearly, however, factors other than the mere presence of coliform organisms must be involved since all Cheddar cheese probably contain these organisms initially.

Observations at three factories have suggested that there are two types of blown cheese. The commoner or normal type of blown factory cheese develops gradually during storage and invariably appears to be associated with the presence of *coli* I strains. Thus, of forty-four blown cheese sampled during storage, forty-three yielded *coli* I strains only. The less common type of gas production is very rapid and usually violent; it occurs within a few days after cheese leaves the press or even in the curd itself. During outbreaks of this type various mixtures of coliform strains were found during manufacture; some data showing the associations met with are given in Table 13. All the strains formed gas in milk, but the *aerogenes* strains showed particularly rapid and copious gas formation. Moreover, an *aerogenes* I strain was present in every case. Nevertheless, within a few weeks *aerogenes* strains could no longer be isolated and only *coli* I strains were found in the cheese.

During these particular outbreaks no coliforms were found in samples of the bulk starter used, and the infection was traced to post-pasteurization contamination of the cheese milk. Kelly(51) states that gassy Limburg cheese may be produced by *coli* or *aerogenes* strains, either singly or together.

(*c*) *Pasteurized Cheddar cheese*

Pasteurized (processed) cheese may sometimes become blown after long storage at high temperatures. This defect has never been traced to the action of coliform organisms and has always been associated with the growth of anaerobic spore-forming bacilli. During such investigations presumptive coliform positives were obtained on twenty-three occasions, but all were false positive reactions produced by the anaerobe concerned.

Plant surfaces

Reference has already been made to coliform contamination from plant surfaces, and a brief summary of the results obtained by direct examination of some such surfaces (Table 4) may be included here.

For reasons previously mentioned (p. 250) plant washing waters frequently contained coliform organisms. The cultures isolated comprised 58·7% *coli* and 34·8% *aerogenes*, but few intermediate-irregular strains.

Rinse waters from plant which had been submitted to a sterilizing process (e.g. pipelines, pasteurizing plant, evaporators) yielded few coliform organisms, but rinses of cream separators and cheese tanks (washed but not steamed) frequently gave positive tests. The main feature which emerged from the detailed tests was the high proportion (75%) of *coli* cultures obtained. Churn rinses rarely yielded positive coliform tests; of the cultures studied 53·0% were *coli* types, but the most unusual feature was the relatively frequent occurrence (29·4%) of intermediate-irregular types.

The plant-swab technique probably provided the best means of studying the coliform flora of plant surfaces. No coliform organisms were obtained from swabs of hot-milk surfaces (in pasteurizing plant), but by examining large volumes of swab solutions coliform-positive tests were infrequently obtained from other plant surfaces, e.g. cheese tanks, cream-storage vats, raw-milk tanks, pumps, filters, coolers, and pasteurized milk balance tanks. The proportion of false positive tests (34·5%) was, however, very high. Of the fifty cultures isolated from plant swabs, twenty-two were *coli*, fifteen *aerogenes-cloacae*, and thirteen intermediate-irregular types (a relatively high proportion). The *aerogenes-cloacae* cultures were isolated only from raw-milk equipment and wooden butter churns.

The heat-resistance data obtained during this study of the coliform flora of plant surfaces have already been discussed on p. 250.

Air tests

In order to study the possibility of coliform infection from atmospheric sources, Petri dishes containing E.M.B. agar were exposed for 30 min. on a number of occasions in various situations of manufacturing plants. A total of seventy exposures provided little direct evidence of atmospheric coliform contamination inside factory premises. No coliform organisms were obtained on the plates with the exception of a few placed in close proximity to cream separators handling raw milk. Fine droplets containing coliform organisms were probably disseminated into the atmosphere around separators and any neighbouring plant might be infected. Of seven cultures studied, three were *coli* I, one *coli* II, and three *aerogenes* I strains.

The distribution of coliform types

In order to present a complete picture of the distribution of coliform types over the whole field of dairying activities, the data relating to all the individual processes are given in condensed form in Table 14. If the sources of the 2508 cultures isolated are divided into only three main groups, the following general distribution is observed:

	No. of cultures	*Coli* %	Intermediate-irregular %	*Aerogenes-cloacae* %
Raw milk	228	55·2	14·5	30·3
Manufacturing processes	2102	52·8	12·25	34·95
Plant surfaces	178	57·8	16·9	25·3
	2508			

This very wide general survey therefore indicated that the *coli* and intermediate-irregular groups occurred most frequently on plant surfaces and least frequently in manufactured products, whilst the *aerogenes-cloacae* group was most abundant in manufactured products and least so on plant surfaces. Apart, however, from the low percentage of *aerogenes-cloacae* cultures isolated from plant surfaces, the variations between the three

Table 14. *Summary of incidence of coliform types in all processes*

Source of cultures	Total cultures		Classification of coliform types: *Coli* I	*Coli* II	Intermediate I	Intermediate II	*Aerogenes* I	*Aerogenes* II	*Cloacae*	Irregular III	Irregular IV	Irregular V	Irregular VIII
Manufacturing processes:													
Holder-pasteurized milk	185	No.	119	21	6	1	23	1	6	1	—	4	3
		%	64·3	11·3	3·2	0·6	12·4	0·6	3·2	0·6	—	2·2	1·6
			75·6		3·8		16·2			4·4			
H.T.S.T.-pasteurized milk	48	No.	20	5	2	—	14	2	2	2	—	—	1
		%	41·7	10·4	4·2	—	29·1	4·2	4·2	4·2	—	—	2·1
			52·1		4·2		37·5			6·3			
Ice cream	56	No.	18	2	8	2	20	2	—	—	—	2	2
		%	32·1	3·6	14·3	3·6	35·7	3·6	—	—	—	3·6	3·6
			35·7		17·9		39·3			7·2			
Milk powder	308	No.	131	12	8	6	88	2	49	1	—	6	5
		%	42·5	3·9	2·6	1·9	28·6	0·6	15·9	0·3	—	1·9	1·6
			46·4		4·5		45·1			3·8			
Condensed milk	222	No.	67	2	4	2	129	4	9	—	—	—	5
		%	30·2	0·9	1·8	0·9	58·1	1·8	4·1	—	—	—	2·2
			31·1		2·7		64·0			2·2			
Pasteurized cream	205	No.	85	10	11	2	60	11	4	10	—	8	4
		%	41·5	4·9	5·4	1·0	29·3	5·4	1·9	4·9	—	3·9	1·9
			46·4		6·4		36·6			10·7			
Clotted cream	98	No.	38	9	3	—	25	6	7	—	1	7	2
		%	38·8	9·2	3·1	—	25·5	6·1	7·1	—	1·0	7·1	2·0
			48·0		3·1		38·7			10·1			
Butter	310	No.	128	16	24	2	99	16	21	2	—	2	—
		%	41·3	5·2	7·7	0·6	31·9	5·2	6·8	0·6	—	0·6	—
			46·5		8·3		43·9			1·2			
Soft cheese	393	No.	225	12	25	6	79	8	5	—	—	11	22
		%	57·2	3·1	6·4	1·5	20·1	2·0	1·3	—	—	2·8	5·6
			60·3		7·9		23·4			8·4			
Hard cheese	277	No.	172	18	31	2	38	2	2	3	—	3	6
		%	62·1	6·5	11·2	0·7	13·7	0·7	0·7	1·1	—	1·1	2·2
			68·6		11·9		15·1			4·4			
Total from manufacturing processes	2102	No.	1003	107	122	23	575	54	105	19	1	43	50
		%	47·7	5·1	5·8	1·1	27·35	2·6	5·0	0·9	0·05	2·0	2·4
			52·8		6·9		34·95			5·35			
Plant washing waters	46	No.	23	4	1	—	14	2	—	—	—	—	2
		%	50·0	8·7	2·2	—	30·4	4·3	—	—	—	—	4·3
			58·7		2·2		34·7			4·3			
Plant rinse waters	48	No.	36	—	—	—	7	1	—	—	—	2	2
		%	75·0	—	—	—	14·6	2·1	—	—	—	4·2	4·2
			75·0		Nil		16·7			8·4			

Table 14 (*continued*)

Source of cultures	Total cultures		Classification of coliform types: *Coli* I	*Coli* II	Intermediate I	Intermediate II	*Aerogenes* I	*Aerogenes* II	*Cloacae*	Irregular III	Irregular IV	Irregular V	Irregular VIII
Plant swabs	50	No.	18	4	10	2	12	2	1	—	—	1	—
		%	36·0	8·0	20·0	4·0	24·0	4·0	2·0	—	—	2·0	—
			44·0		24·0		30·0			2·0			
Churn rinses	34	No.	11	7	5	—	4	2	—	1	—	2	2
		%	32·35	20·6	14·7	—	11·8	5·9	—	2·9	—	5·9	5·9
			53·0		14·7		17·7			14·7			
Total from plant surfaces	178	No.	88	15	16	2	37	7	1	1	—	5	6
		%	49·4	8·4	9·0	1·1	20·8	3·9	0·6	0·6	—	2·8	3·4
			57·8		10·1		25·3			6·8			
Raw milk	228	No.	118	8	12	7	57	5	7	—	—	9	5
		%	51·7	3·5	5·3	3·1	25·0	2·2	3·1	—	—	3·9	2·2
			55·2		8·4		30·3			6·1			

main groups were remarkably small. Nevertheless, the detailed data summarized in Table 14 show clearly that considerable differences were found between individual dairy products and processes. These may conveniently be discussed in relation to the ecological distribution of individual coliform types.

(1) Coli *group*

The *coli* types were undoubtedly the most abundant coliform organisms found throughout all branches of dairying. They were particularly abundant in pasteurized milk, hard cheese, and soft cheese, and occurred frequently on plant surfaces and in raw milk. The type I always outnumbered type II strains in most products by a considerable margin; among the total collection of 1339 *coli* cultures the ratio of type I : type II was 9 : 1. Type II strains were relatively frequent in churn rinses, pasteurized milk, bulk cream, and clotted cream.

(2) *Intermediate group*

Intermediate strains comprised only a small proportion of the general coliform flora, viz. 8·4% of cultures from raw milk, 6·9% from manufactured products, and 10·1% from plant surfaces. Their main general source appeared to be the surfaces of factory plant, and they were associated most prominently with churn rinses, milk-bottle rinses, plant swabs, ice cream, butter, soft cheese, and the early stages of hard-cheese manufacture. The type I strains were more numerous than type II strains, a ratio of 4·7 : 1 being obtained from 182 cultures. Intermediate II strains were decidedly uncommon and comprised only 1·1% of the coliform cultures obtained from manufactured products and plant surfaces; in some products they were never found.

(3) Aerogenes-cloacae *group*

Although rather less common than the *coli* group, the *aerogenes-cloacae* group occurred very frequently throughout the range of cultures examined, and was particularly abundant in the collections isolated from condensed milk, milk powder, and butter. The group was

apparently least common in holder-pasteurized milk (unless operating conditions were very poor), in cheese, and in plant swabs and churn rinses. Of the three types, *aerogenes* I was markedly predominant, followed by *cloacae* and *aerogenes* II in roughly equal numbers, although the *cloacae* figures were heavily weighted by the unusually frequent occurrence of this strain in milk powder. The total collection of 848 cultures yielded an *aerogenes* I : *cloacae* : *aerogenes* II ratio of 10 : 1·7 : 1. The *aerogenes* II strains were isolated most commonly from butter, cream and H.T.S.T.-pasteurized milk, whilst the *cloacae* strains were most noticeable in milk powder, clotted cream and butter; the *aerogenes* II strain was much more common on plant surfaces.

(4) *Irregular group*

Only about 5–6% of the cultures studied were classed as irregular types. The occurrence of these organisms seemed to be largely fortuitous excepting in bulk cream, clotted cream, and churn rinses, and possibly in soft cheese and plant rinses; from these sources significant numbers of irregular cultures were isolated. The factory operations involved in packing bulk and clotted cream were outstanding sources of irregular types, but they were rarely found in butter or condensed milk, in which products packing operations are also responsible for much of the coliform flora. Only one irregular IV culture was isolated, and omitting this the total of 138 irregular cultures comprised types III, V, and VIII in a ratio of 1 : 2·8 : 3·0. Types V and VIII were therefore the most important irregular coliforms, of which type VIII was probably rather more common. Irregular III was isolated least frequently of all the coliforms, and was not found at all in raw milk, plant swabs, condensed milk, ice cream, clotted cream, or soft cheese.

Factors affecting distribution of types

The ecological factors which influence the coliform flora of different dairy products have not been elucidated in detail, but among them the composition of the product and the effects of heating and drying are obviously important.

Heat resistance

During this investigation heat-resistance experiments were performed on cultures which had been isolated on laboratory media and were subsequently heated in milk at 63° C. for 30 min. Other workers have adopted similar methods, but Wilson(1) heated cultures in heart broth at 60° C. for 30 min. It has been shown (p. 250), however, that at least in the case of raw milk this method yielded a considerably higher proportion of heat-resistant cultures than was obtainable by direct pasteurization of raw-milk samples. Repeat heat-resistance tests on laboratory cultures usually confirmed the original result and seemed to indicate that the presence or absence of some heat-resistant cells was a constant characteristic of the cultures. Elliker & Frazier(26), who determined heat resistances at 53° C. for 30 min., found that heat resistance reached a minimum value during the period of maximum reproduction and a peak value at the commencement of the stationary growth phase; moreover, incubation of cultures either below or above their optimum temperature also affected the results.

Further work is obviously desirable, but the data obtained from heat-resistance tests on 913 cultures (Table 15) are of some interest. Whilst the incidence of heat-resistant coliforms might have been due to a few cells present in laboratory cultures and in excess

of the true values for the original products themselves, the figures probably indicate the relative tendencies to develop heat-resistant cells given favourable circumstances.

The data show significant variations in the ability of different coliform types to produce heat-resistant cells. The most heat-resistant strain was undoubtedly *coli* I; 406 cultures yielded 51% of heat-resistant strains. The intermediate II strain also gave eight resistant cultures out of eighteen and was possibly a second highly resistant form. The weakest

Table 15. *Incidence of heat-resistant coliform strains*

	Coli				Intermediate				*Aerogenes*			
	I		II		I		II		I		II	
Source of strains	T	HR	T	HR	T	HR	T	HR	T	HR	T	HR
Raw milk	26	5	4	—	7	—	4	1	15	—	2	—
Pasteurized milk	52	22	15	1	7	1	1	1	10	3	3	—
Ice cream	5	2	1	—	4	1	1	—	6	—	—	—
Milk powder	74	41	5	2	4	—	4	4	38	11	2	1
Condensed milk	23	15	2	2	—	—	2	—	51	9	3	—
Pasteurized cream	8	2	2	2	2	—	—	—	24	8	2	—
Butter	40	19	6	1	20	2	1	—	27	3	6	1
Soft cheese	66	42	2	1	11	—	2	—	23	8	2	2
Hard cheese	50	22	6	1	12	—	—	—	11	3	—	—
Plant washing waters	13	7	3	—	1	—	—	—	10	6	2	2
Plant rinse waters	26	18	1	—	—	—	—	—	2	—	1	1
Plant swabs (raw milk)	5	1	2	1	3	1	1	—	4	—	—	—
Plant swabs (pasteurized milk)	8	6	2	—	2	—	2	2	—	—	2	—
Churn rinses	10	5	7	1	5	1	—	—	4	1	2	1
Total all sources	406	207	58	12	78	6	18	8	225	52	27	8
Percentage		51·0		20·7		7·7		44·4		23·1		29·6

			Irregular								
	Cloacae		III		V		VIII		All strains		
Source of strains	T	HR	T	HR	T	HR	T	HR	T	HR	% HR
Raw milk	4	—	—	—	3	—	1	—	66	6	9·1
Pasteurized milk	10	4	1	1	4	2	1	1	104	36	34·6
Ice cream	—	—	—	—	1	—	1	—	19	3	15·8
Milk powder	17	2	—	—	4	—	3	—	151	61	40·4
Condensed milk	1	—	—	—	—	—	2	—	84	26	30·9
Pasteurized cream	—	—	10	—	2	2	2	—	52	14	26·9
Butter	6	1	—	—	—	—	—	—	106	27	25·5
Soft cheese	2	1	—	—	8	1	3	—	119	55	46·2
Hard cheese	—	—	1	—	1	—	1	—	82	26	31·7
Plant washing waters	—	—	—	—	—	—	2	2	31	17	54·8
Plant rinse waters	—	—	1	—	2	2	1	1	34	22	64·7
Plant swabs (raw milk)	—	—	—	—	1	—	—	—	16	3	18·8
Plant swabs (pasteurized milk)	—	—	—	—	—	—	—	—	16	8	50·0
Churn rinses	—	—	1	—	2	1	2	1	33	11	33·3
Total all sources	40	8	14	1	28	8	19	5	913	315	34·5
Percentage		20·0		7·1		28·6		26·3			

T = total strains examined. HR = number of strains which survived heating in milk at 145° F. for 30 min.

tendency to produce heat-resistant cells (about 7%) was exhibited by intermediate I and irregular III cultures. Of the intermediate tendencies, *coli* II, *aerogenes* I, and *cloacae* cultures gave heat resistances between 20 and 23%, whilst *aerogenes* II, irregular V, and irregular VIII showed rather higher figures between 26 and 29%. Of the total of 913 cultures 34·5% produced heat-resistant cells, but this figure was influenced by the fact that 406 (44%) of the cultures examined were *coli* I types; nevertheless, it should be borne

in mind that *coli* I types comprised 52% of all cultures from raw milk and 48% from manufactured products.

Other workers have observed the frequent resistance to heating of *coli* I strains, but it is interesting to note that other coliform types may on occasion exhibit similar characteristics. Hammer & Hussong(23) found that old *aerogenes* cultures obtained from ropy milk sometimes resisted heating at 63° C. for 20 min. The data in Table 15 reveal very considerable variations in the proportions of heat-resistant coliform cultures isolated from different sources, ranging from 64·7% (plant rinse waters) down to 9·1% (raw milk). The heat-resistance figures were naturally influenced by the proportion of *coli* I strains in each particular collection of cultures, but the relationship was not completely parallel. In particular, raw milk and raw-milk plant yielded a much lower proportion of heat-resistant cultures than did manufactured products.

The temperature conditions prevailing during particular processes have already been discussed, and it seems clear that such conditions may be a major factor determining the predominant coliform types associated with individual products and processes. When a process involves prolonged exposure to high temperatures (approaching 145° F.) a predominance of *coli* I strains may be expected.

Other factors

The influence of drying as a factor is only important in the particular case of milk powder; it has been suggested (p. 255) that spray drying favours the survival of *cloacae* and *aerogenes* strains at the expense of *coli* strains.

The composition of some products may influence the nature of their coliform flora. Thus the sugar content of condensed milk and ice cream and the salt content of butter probably lead to selective survival of *aerogenes-cloacae* strains, whilst fatty materials such as liquid or clotted cream may possibly provide a favourable substrate for irregular types. The conditions associated with cheese-making appear to favour *coli* types (these may represent a combined effect of temperature, chemical and physical changes in the material), whilst intermediate types show a distinct tendency to survive in residual moisture present on plant surfaces, churns, and milk bottles.

Finally, it may be repeated that coliform contamination was frequently associated with ancillary rather than main processes, e.g. post-pasteurization contamination (milk and ice cream) and packing into containers (cream and condensed milk).

The value of the presumptive coliform test

The value of the presumptive coliform test in the bacteriological examination of milk and milk products may now be discussed from several aspects.

(1) *Sources of error*

The experimental errors which may arise when the test is applied to raw-milk examinations have been discussed (p. 235), and some evidence has been given on p. 239 which suggests that if suitable precautions are taken these particular errors do not assume serious proportions when manufactured products are examined. The predominance of *coli* types recorded in the foregoing data may partly have been due to selective cultivation of such types in the MacConkey broth medium, but, nevertheless, many other coliform types were also isolated whilst mixed cultures were of common occurrence. Indeed, the

purification of mixed cultures proved to be the major practical difficulty associated with confirmatory tests.

During this investigation the error arising from false-positive presumptive tests (due to extremely heat-resistant spore-forming organisms) appeared to be of considerable significance in the case of some particular products. The danger of false-positive presumptive reactions was negligible when the test was applied to raw-milk samples; this observation agreed with the findings of Wilson (1). In addition, only a small proportion of false reactions was obtained from butter (7·1%), soft cheese (8·0%), and Cheddar cheese (5·6%). A very high incidence of false reactions was recorded for processed cheese (100%), milk powder (51·9%), churn rinses (45·4%) and plant swabs (34·5%), whilst quite high figures were obtained from heavy inoculations (10 ml.) of clotted cream (24·2%) and holder-pasteurized milk (19·8%).

False positives occur most frequently when large inoculations of material are employed, and when this procedure must be followed confirmatory tests become essential if accurate conclusions are to be drawn; provided this requirement is recognized the utility of the coliform test need not be invalidated. Confirmation of the mere presence or absence of a coliform organism, without proceeding to a detailed identification of the particular type, is not unduly laborious unless the number of samples involved is very large. The alternative procedure of examining smaller quantities of material merely results in frequent failure to detect coliform organisms in samples containing very small numbers. Such considerations particularly apply to the examination of milk powder, swabs and rinses of equipment, and some special samples such as pasteurized milk or cream taken directly from heating sections of pasteurizing plants, and laboratory-pasteurized samples of raw milk.

False-positive reactions produced by yeasts were of little practical significance. Only thirty-one instances were recorded, of which fourteen were obtained from soft cheese, eight from condensed milk, seven from butter, one from Cheddar cheese, and one from clotted cream.

It has been shown (pp. 256, 259, 271) that coliform organisms gradually die out in milk powder, condensed milk, and Cheddar cheese when these products are stored; consequently, coliform tests are only of value when applied to freshly made samples, and negative coliform tests obtained from samples of unknown age are of little significance. A further complication arises from the increasing incidence of false-positive tests as the age of samples of these three products increases, and this possibility should always be borne in mind.

(2) *Public health aspects*

Wilson (1) has pointed out that none of the premises upon which the application of the coliform test to water analysis is based is valid for raw milk. A considerable proportion of the coliform organisms present in raw milk may not be true intestinal *coli* types, whilst even the true *coli* types provide no evidence of direct excretal pollution since they may be derived from unsterile utensils as well as from cow dung; moreover, rapid multiplication of coliform organisms may occur in raw milk prior to sampling and testing, which may result in a misleading picture of the original contamination. Wilson also stresses the fact that coliform organisms in themselves are not dangerous, but merely serve as an index of the possible presence of pathogenic organisms.

In the case of manufactured milk products, the arguments against this interpretation

of the test are even stronger. The frequency of *coli* types in particular products may be greater or less than in raw milk—on a broad average it is probably rather less. In any case, the *coli* types found in manufactured products are manifestly not derived from excretal pollution, which is almost impossible; rather, as has been shown, are they a reflexion of adaptation to particular conditions associated with each product which may favour their survival. Moreover, it must be remembered that the coliform flora of manufactured dairy products is rarely derived directly from the raw-milk supply. The errors due to bacterial multiplication between the times of production and testing, which limit the value of the coliform test on raw milk, may also apply to manufactured products when testing is delayed; in some products coliform organisms will multiply rapidly whilst a decrease may occur in others (e.g. milk powder, condensed milk, salted butter). This difficulty can be greatly reduced in plant-control laboratories where it is usually possible to perform the coliform test within a short time after manufacture, whereas it is rarely possible to test raw milk at the point of production.

Clearly, however, the particular interpretation of the coliform test which is based upon danger to public health is unsound and should be abandoned. Acceptance of this viewpoint does not, as is often assumed, completely invalidate the coliform test; on the contrary, it becomes easier to assess the true value of the test.

(3) *Plant-control aspects*

The coliform test is undoubtedly one of the most valuable plant-control methods at present available. To recapitulate a few of the examples which have already been discussed, it may be used to detect post-pasteurization or bottling contamination of milk, post-heating infection of ice cream, pre-drying contamination in milk-powder manufacture, canning contamination of condensed milk, and unsatisfactory handling of cream, or as a check upon the cleanliness of butter and cheese equipment. In many instances the total colony count may be very small and fail to reveal slightly faulty methods which are readily detected by the coliform test; in others the colony count may be appreciable but composed in part of thermoduric or thermophilic organisms. The test is probably less useful when applied to the examination of plant swabs or churn rinses, which may show undesirable numbers of bacteria when coliform organisms are absent.

For research purposes confirmatory tests and heat-resistance experiments appear to be desirable, otherwise useful information may be missed. In many cases it is at least essential to ensure that true positives are recorded in order to avoid completely false conclusions. For routine control purposes the presumptive test should provide all the information required. Differentiation of 'intestinal types' is of no value from a public health standpoint and the only precautions needed are (1) elimination of false-positive reactions, and (2) reduction to a minimum of any possible bacterial growth in the samples prior to testing. The fact that some coliform strains may develop partial heat resistance is probably of little importance in plant-control practice. It is believed that instances wherein heat-resistant strains are present in appreciable numbers in the raw milk and subsequently survive a heating process are uncommon. On the other hand, it has been shown that heat-resistant coliforms may occur commonly as a result of post-heating contamination. Moreover, it is suspected that heat-resistance determinations are subject to variations which depend upon the conditions of experiment; the interpretation of such results probably requires caution pending further extensive investigation.

(4) *Economic aspects*

So far this discussion has been confined to the use of the coliform test as a basis for assessing the degree of efficiency attained in dairy processing, but important economic aspects may also be involved since deleterious changes result from the growth of coliform organisms in many dairy products. From this latter standpoint two groups of dairy products may be distinguished.

In the first group of products coliform organisms may be unable to multiply, but in any case their presence has no apparent relation to deterioration of quality. Such products include milk powder, condensed milk, and soft cheese, and in many cases salted butter and Cheddar cheese probably fall into this category. In the second, and much larger group, coliform organisms are harmful in themselves and are frequently responsible for undesirable changes. Examples include souring and objectionable flavours in raw milk, pasteurized milk, ice cream, liquid cream, and clotted cream, the decomposition of unsalted butter, and gassy fermentation of hard cheese. The presumptive coliform test is obviously of direct value as a control method of preventing or delaying such changes. For research purposes further differentiation of types is usually desirable.

SUMMARY

1. The sources of error and general significance of the presumptive coliform test have been studied, with special reference to manufactured dairy products.

2. Eosine methylene blue agar has been found reasonably satisfactory for isolation of cultures but unsatisfactory as a direct plating medium.

3. A wide survey of the coliform flora of raw milk and milk products has been made over a period of 11 years. The data were collected from 24,952 samples and include the incidence of presumptive positive tests at each stage of manufacturing processes, the occurrence of 'false-positive' tests, the detailed distribution of coliform types in individual dairy products, and some preliminary observations on 'heat-resistant' coliform strains.

4. Material examined included raw milk, pasteurized milk, ice cream, milk powder, condensed milk, pasteurized cream, clotted cream, butter, soft cheese, Cheddar cheese, processed cheese, swabs of dairy plant, churn rinses, and the atmosphere of dairy factories.

5. Detailed confirmatory tests were performed on 2490 presumptive positives, from which 2508 coliform cultures were isolated and classified.

6. A general ecological survey of the frequency of individual coliform types in dairying operations as a whole has been attempted.

7. Particular factors which may affect the distribution of coliform types in specific products or situations have been shown to include heat resistance, resistance to drying, chemical composition of the substrate, the effect of storage, and conditions prevailing on plant surfaces. It is suggested that the coliform flora may be partly the result of adaptation to conditions associated with each individual product.

8. The value of the presumptive coliform test has been discussed in respect of public health requirements, as a plant-control method, and in relation to economic aspects. The test is believed to be of great utility in the plant-control laboratory but of little value for public health purposes.

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334. A SIMPLE AGAR MEDIUM FOR THE GROWTH OF LACTIC STREPTOCOCCI: THE ROLE OF PHOSPHATE IN THE MEDIUM

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(With 1 Plate)

A prime requisite of bacteriological research is a simple and reproducible artificial medium on which to isolate and maintain pure cultures of the different species of bacteria. For the propagation of lactic acid bacteria, milk, plain or enriched, has proved to be a convenient fluid medium, while modifications of the common peptone and so-called 'digest' solid media have been used by individual workers in the study of particular organisms.

In New Zealand, pure cultures of lactic streptococci have been widely adopted for cheese making, and it has been the practice to select organisms which are active acid formers at temperatures ranging from 20 to 37° C. The cultures are transferred daily in sterilized skim milk and repurified at regular intervals by selection of one of the more active colonies from a solid medium. In this way the activity of cultures has been maintained for long periods of time; at least four cultures have been subcultured daily at this Institute and supplied to commercial dairy factories for the past 10 years. They are as active to-day as when originally isolated. In earlier years, an agar medium containing milk-whey, peptone, and yeast extract (yeast-whey agar) was employed, and during the plating procedure the curious anomaly was encountered that certain vigorous strains frequently yielded only a small proportion of colonies which attained the activity of the parent culture. In some strains changes in acid-producing power were sometimes accompanied by changes in colony form (1), but in the majority of strains the differences were not detectable until the colonies were picked into milk. An undesirable feature, however, was the variability of results obtained with different batches of solid medium on which the cultures were streaked. This lack of uniformity in the medium was attributed partly to variations in the technique of preparation from time to time, and partly to differences in the composition of the whey obtained by enzymatic digestion of different milks for varying periods of time. The necessity for several filtrations at various stages appeared to contribute further to variations in the growth-promoting value of the final medium. Inability to control variation in the relatively complex medium, and, more especially, the frequent production of a medium yielding only a poor growth of small and often abnormal colonies of low vitality led to a search for a simpler and more reproducible solid medium suitable for the cultivation of the various streptococci associated with milk and dairy products. In work of this nature it is important to obtain rapid-growing normal colonies of maximum size and activity, and, for convenience, it is also desirable that the selected medium be readily prepared from more or less standardized commercial nutrient materials, so that uniform cultural conditions may be obtained over an extended period.

Trials with media of different composition

During the course of early experiments it was noted that a meat extract-lactose-peptone combination gave results far superior to those obtained on many batches of the more complicated yeast-whey agar, and a series of preliminary trials showed that a medium of

the following composition contained most of the nutrients required by the lactic streptococci: agar, 2%; beef extract, 1%; lactose, 2%; peptone, 1%; yeast extract, 10%; NaCl, 0·5%; *p*H, 7·0. When a very small loopful of a 1 : 10 dilution of a 24 hr. clotted milk culture of the test organism (*Str. cremoris* and *Str. lactis* strains) was streaked over the well-dried agar surface and the plates incubated at 22° C. for 48 hr., the unfiltered medium regularly gave a good uniform growth of smooth, convex, 'enamel drop' colonies about 0·5 mm. in diameter. The clear medium obtained by filtration through paper pulp was definitely less satisfactory than the unfiltered medium. It gave growth which could only be rated as fair to fairly good, yielding a large proportion of abnormal (i.e. abnormal in size, shape and texture) and sometimes slow-clotting colonies. A representative set of results is given in Table 1. Since 'abnormal' colonies commonly required a relatively longer time to clot milk, only 'normal' well-isolated colonies were selected for comparison from plates containing a mixture of types. In all cases the whole colony was transferred to tubes containing 10 ml. sterilized skim milk.

Table 1. *Growth of* Str. cremoris *(strain HP) on filtered and unfiltered media. Milk-clotting power of colonies picked off. Temperature* 22° C.

Medium	Batch	Growth. Colony types after 48 hr.	% milk tubes clotted within 20 hr.	24 hr.	30 hr.
Unfiltered	1	Good. Colonies normal	30	90	—
	2		0	80	—
	3		70	100	—
	4		0	100	—
	5, 6, 7 and 8		100	—	—
Filtered	1	Fair. A few abnormal colonies	0	0	100
	2	Fairly good. A few abnormal colonies	0	40	100
	3	Fairly good. Some abnormal colonies	0	0	55
	4	Fairly good. Some abnormal colonies	0	0	0
	5	Fair. Some abnormal colonies	0	0	20
	6	Fair. Some abnormal colonies	0	0	100
	7	Poor. Some abnormal colonies	0	0	20
	8	Poor. Some abnormal colonies	0	0	0

Filtration was further shown to result in (1) loss in nitrogenous materials—average figures for T.N. on different batches: unfiltered 0·23%, filtered 0·16%, i.e. a 30% loss—and (2) loss of buffering substances from the medium equivalent to a difference averaging 0·5 ml. in the quantity of *N* acid required to change the hydrogen-ion concentration of 100 ml. of the filtered and unfiltered medium from the initial *p*H to *p*H 5·0.

The aseptic addition of skim milk, whey, casein, peptone and beef extract gave considerable improvement in growth rate, colony type and size, as well as an increased number of colonies capable of clotting milk in less than 24 hr. In no instance, however, did any addition produce a result strictly comparable with unfiltered controls.

Since filtration had such a marked depressing effect on the growth-promoting properties of the medium, and since, moreover, the medium could be used quite conveniently with the precipitate not removed, the filtration process was omitted during subsequent experiments. In an attempt to ascertain which of the four constituents (apart from the lactose and agar) were really essential for growth, a series of media were prepared, each of the constituents being omitted in turn. Plating of a strain of *Str. cremoris* on these media, picking of normal colonies after incubation for 48 hr., and observation of the milk-clotting powers of the colonies, led to the following conclusions.

(*a*) *Salt.* The inclusion of 0·5% NaCl in the medium had no effect. Evidently the salt present in the beef extract was ample for growth requirements.

(*b*) *Yeast extract.* The yeast extract had no significant influence on the growth of *Str. cremoris*. Since, however, any medium used in dairy work may on occasion be used for the cultivation of betacocci and betabacteria which are known to be stimulated by yeast extract the extract was still included in the medium.

(*c*) *Beef extract.* The beef extract was important. Its omission led to a very much poorer growth with abnormal types of colony. Even the normal colonies showed a rather slow milk-clotting action. The extract probably acts in two ways. It supplies a considerable part of the available nitrogen in the medium and also serves as a source of B-vitamins. Analysis of one batch of extract showed riboflavin 3·2 mg.%, nicotinic acid 80 mg.%, thiamine 0·06 mg.%, when assayed by the microbiological methods of Snell & Strong (2), Snell & Wright (3) and Sarett & Cheldelin (4) respectively.

(*d*) *Peptone.* Peptone was important as a source of part of the available nitrogen. Its omission gave results similar to those obtained when beef extract was omitted. There was, however, a further effect connected with the peptone fraction of the medium which became apparent when different brands of peptone were tested. Although all peptones gave media which produced a majority of colonies capable of clotting milk within 24 hr. at 22° C., the colony size on different media in 24 and 48 hr. varied considerably. Previous workers have shown that commercial peptones differ greatly in the types of nitrogen compound they contain, and that because of their content of certain nitrogen fractions some peptones are better suited than others for the growth of particular organisms (5, 6, 7).

The lactic streptococci seem to give speedier growth and hence larger colonies within 24 hr. when the peptone in the medium is one which contains a high percentage of the more complex nitrogen fractions.

Effect of added buffer

At this stage in the investigation it was observed that growth of streptococci on the surface of the full agar medium containing lactose, yeast extract, peptone and beef extract rapidly lowered the *p*H of the medium from 7·0 to 5·0. This suggested a need for the addition of buffer salts and phosphate was tried. The effect was outstanding. When sodium phosphate at *p*H 7·0, sufficient to give *M*/20 concentration in the whole medium, was used, it resulted in more rapid growth, much larger colonies (0·7–1·0 mm. in diameter after 48 hr.) and a shortening of the milk-clotting times of the colonies picked from the medium. The effect was obviously due in part to maintenance of the medium within a *p*H range suitable for the most active growth of the streptococci although, as will be shown later, there is probably a separate growth-stimulating action due to incorporation of phosphate in the medium. The change in *p*H of the phosphate medium after streptococci had grown for 48 hr. upon it was from *p*H 7·0 to 6·0 instead of to 5·0 with the unbuffered medium. This corresponded to the increased buffered capacity found on examination. (Number of ml. of *N* acid required to change 100 ml. of the medium from *p*H 6·8 to 5·0: buffered 4·8, unbuffered 2·1.) It seems surprising that the need for effective buffering in a medium in which acid is to be formed had not received adequate consideration before, but it is a fact that in relatively few published recipes for media intended for the growth of acid-producing organisms is there adequate provision for maintenance of the *p*H at as stable a figure as is reasonably possible.

Reverting for a moment to the relative values of different peptones, an experiment carried out after the discovery that phosphate was of such outstanding value in the medium is worthy of record. Extent of growth and colony size of a strain of *Str. cremoris* was observed on eight agar media containing different peptones plus lactose, yeast extract, beef extract and phosphate. The results are given in Table 2. The results indicate that five of the peptones give almost equivalent results in 48 hr., with three peptones somewhat less stimulating in action. Difco Neo-peptone, which is said to have toxic elements removed from it during preparation (8), gave the greatest stimulation resulting in very early growth. Since, however, we usually incubate for 48 hr. before picking colonies it did not appear necessary in subsequent experiments nor in our routine work to change from the B.D.H. peptone of which alone we had ample stocks.

Table 2. *Growth of* Str. cremoris (*strain HP*) *on solid media containing different peptones. Incubation temperature* 22° C.

	24 hr.		48 hr.	
Peptone	Growth	Average colony size (mm.)	Growth	Average colony size (mm.)
Bacto-peptone, Difco	±	—	+ +	0·5
Proteose-peptone, Difco	+ + +	0·25	+ + + +	1·0
Neo-peptone, Difco	+ + + +	0·5	+ + + +	1·0
Bacto-tryptose, Difco	+ + +	0·2	+ + + +	0·9
Bacto-tryptone, Difco	+ + +	0·25	+ + + +	1·0
Peptone, B.D.H.	+ + +	0·25	+ + + +	0·9
Peptone, Witte	+	0·12	+ + + +	0·65
Peptone, Parke Davis	+ + +	0·25	+ + + +	0·75

Composition of medium finally adopted

The foregoing trials now permitted the formulation of a medium which gives the best growth results with lactic streptococci which we have so far observed. The composition is as follows:

Agar	2%
Beef extract	1%
Lactose	2%
Peptone	1%
Yeast extract	10%
Disodium phosphate ($Na_2HPO_4\,2H_2O$)	0·6%

(Yeast extract: 1 lb. bakers' yeast to 700 ml. distilled water, steamed 1 hr., stand, decant and centrifuge, autoclave.)

The ingredients are dissolved in distilled water and steamed for 30 min. The *p*H is 6·8 without adjustment. The medium is tubed in 15 ml. quantities and autoclaved at 15 lb. pressure for 20 min.

It will be evident that the preparation is simple and quickly carried out. The medium as finally obtained contains a flocculent precipitate consisting principally of Ca and Mg phosphates. The precipitate settles to the bottom of the tubes and may be left in the tube when the melted medium is decanted into a Petri dish, or it may be evenly distributed through the agar by twirling the tube of melted agar when it gives a slight cloudiness throughout the medium. If desired the precipitate may be conveniently removed from the bulk medium before tubing by centrifugation, sedimentation or filtration through

non-absorbent material such as sintered glass. Removal of the precipitate by filtration through paper pulp presents difficulties which will be dealt with later.

Over a period of two years the medium has been used not only for the isolation of lactic streptococci for cheese starter but for the cultivation of the majority of the types of bacteria associated with dairy products. Table 3 gives an indication of the growth obtained with a variety of lactic acid bacteria, mastitis streptococci, and some pathogenic streptococci.

Table 3

Organism	Temp. ° C.	24 hr. Growth	24 hr. Average diameter colonies (mm.)	48 hr. Growth	48 hr. Average diameter colonies (mm.)
STREPTOCOCCI:					
Str. viridans (Lab. stock)	37	+	0·11	+ +	0·23
Haemolytic *Streptococcus*: 1 (Lab. stock)	37	+ +	0·24	+ + +	0·42
2 (Lab. stock)	37	+ +	0·25	+ + +	0·37
Str. faecalis 2703 (Reading (9))	37	+ + + +	0·72	+ + + +	0·80
'Black' (Reading (9))	37	+ + + +	1·00	+ + + +	1·10
'*Enterococcus*' (Lab. stock)	37	+ + + +	0·64	+ + + +	0·87
Str. lactis (Lab. stock)	30	+ + + +	0·60	+ + + +	0·67
Str. cremoris (Lab. stock)	30	+ +	0·25	+ + + +	0·60
'Non-acid' *Streptococcus* 9S (Lab. stock)	30	+ +	0·21	+ + +	0·45
Mastitis *Streptococcus*: P (Lab. stock)	37	±	—	+ + + +	0·40
Px (Lab. stock)	37	±	—	+ + + +	0·30
R (Lab. stock)	37	±	—	+ + + +	0·35
STREPTOBACTERIA:					
Sbm. plantarum: Type A (Sherwood) (10)	30	—	—	± (+ + + 72 hr.)	– (0·33 72 hr.)
Type B (Sherwood)	30	±	—	+ + + +	0·79
Type C (Sherwood)	30	±	—	+ + + +	0·60
Type D (Sherwood)	30	±	—	+ + + +	0·80
L. arabinosus A.T.C. 8014	30	+ + +	0·26	+ + + +	0·60
Sbm. casei: Type A (Sherwood)	30	±	—	+ + + +	0·30
Type B (Sherwood)	30	±	—	+ + + +	0·38
L. casei A.T.C. 7469	30	+ + +	0·30	+ + + +	0·53
BETABACTERIA:					
Strain No. 1.2 (Sherwood)	30	±	—	+ + +	0·30
L. fermentum A.T.C. 36, 9338	30	—	—	+ + +	0·52
BETACOCCI:					
Strain A (Lab. stock)	30	+ + +	0·37	+ + +	0·62
Strain B (Lab. stock)	30	—	—	+	0·18

DIFFICULTIES ENCOUNTERED IN FILTRATION OF THE MEDIUM

Although for most purposes decantation of the agar medium from the precipitate in the tubes or use of the medium in a slightly cloudy state is no disadvantage, it is desirable on some occasions (e.g. for demonstration of bacteriophage plaques) to have a water-clear medium. Unfortunately, when the phosphate medium was filtered through paper pulp during its preparation (i.e. immediately before the final filling into tubes), growth on the clear medium was very poor (Pl. 1, fig. 1). Normal isolated colonies were rarely obtained after 48 hr. incubation. The effect of filtration on the medium was very much greater than it was on the earlier medium (Table 1) without phosphate.

Removal of the precipitate by centrifugation gave a clear medium equal in growth-promoting power to the unfiltered medium, and addition of paper pulp or extracts of paper pulp to the unfiltered medium did not show any growth-inhibiting action. Therefore it was clear that filtration through paper pulp removed a growth-promoting factor from the medium.

Heating of each of the ingredients of the medium separately with a phosphate solution showed that the agar-phosphate mixture was responsible for the bulk of the precipitate, and it appeared reasonable therefore to eliminate the precipitate by heating the agar-phosphate mixture alone, filtering off the precipitate through paper pulp and then to add the remaining constituents to complete the preparation of the medium. When this was tried the rather surprising fact emerged that the medium so prepared was no better in growth-promoting properties than the medium which was filtered after all the ingredients had been added.

It thus became evident that the agar-phosphate mixture supplied some growth-promoting factor removed by filtration through paper pulp, and that the effect of phosphate in the medium was not solely a buffering effect. Various trials were made in an effort to elucidate the position with results which may be summarized as follows:

I. Agar-phosphate solution at *p*H 8·0–8·2 gave a heavy precipitate when steamed 30 min.:

(1) Removal of the precipitate by centrifugation or sedimentation and addition of the other ingredients to the clear fluid gave an excellent medium, as good as anything so far obtained.

(2) Removal of the precipitate by filtration through paper pulp and addition of the other ingredients to the filtrate gave a very poor medium.

(3) Decantation of the clear fluid from the agar-phosphate mixture, filtration of this clear fluid through paper pulp and addition of the other ingredients gave a very poor medium.

II. Adjustment of the agar-phosphate mixture to *p*H 7·0 or 6·2 from its original reaction of *p*H 8·0–8·2 before the heating treatment and filtration through paper pulp gave a slightly better result in the final medium, but growth was still poor as compared with that in unfiltered medium.

III. Filtration of the phosphate solution alone (*p*H 8·4) through pulp followed by addition of agar and the other ingredients to make an unfiltered medium gave an excellent result, showing that phosphate did not extract any inhibitory substance from the pulp.

IV. Filtration of a water solution of agar (*p*H 6·8) through pulp followed by addition of phosphate and the other ingredients to make an unfiltered medium also gave an excellent result.

V. An agar solution in caustic soda at *p*H 8·0 gave only a small precipitate when steamed for 30 min. Filtration through pulp followed by addition of the other ingredients, including phosphate and adjustment to *p*H 6·9, gave an excellent medium.

VI. Acid washing of a pulp filter (which reduced its *p*H from 8·0–8·2 down to 7·0) before its use for filtering the complete medium did not give any improvement in growth. The filter still extracted some factor from an agar-phosphate mixture.

VII. An agar-phosphate mixture added to a filtered medium showing very poor growth restored the growth-promoting properties. Trace additions were not effective; the growth-promoting value of the final medium was in proportion to the amount of agar-phosphate mixture added.

The results summarized above can be interpreted only on the assumption that agar plays a part in the nutrition of lactic streptococci when they are grown on the medium described. It is generally believed, of course, that with most bacteria agar is merely an agent for rendering a medium solid. Agar consists largely of polysaccharides (galactans

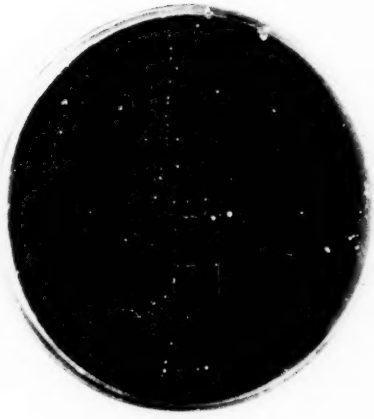

Fig. 1. Filtered medium.

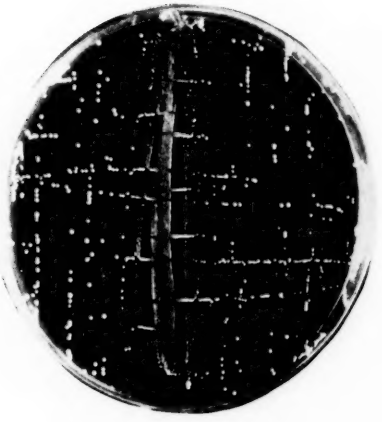

Fig. 2. Unfiltered medium.

Photo: L. Barrett, Massey Agricultural College

Figs. 1, 2. Growth of *Str. cremoris* (strain HP) on the agar medium containing added phosphates. Incubation temperature 22° C. Time 48 hr.

and pentosans). Fellers(11, 12) found it to contain considerable amounts of protein and other soluble ingredients and showed that part of this nitrogen and possibly some of the other nutrients may serve as food for micro-organisms in media containing agar. In particular, he found that part of the protein may be transformed to NH_3 by certain organisms under favourable conditions, although the nitrogenous materials could be utilized to a very limited extent under ordinary conditions.

Whatever may be the actual substances responsible in the present instance it is clear that some component of the agar combines with or is changed by the action of the phosphate at 100° C. to give a growth-promoting substance for lactic acid bacteria. That changes in the chemical composition of the agar take place is evident from the fact that the agar-phosphate mixture assumes on heating a light brown colour with a pinkish tinge as compared with the clear whitish colour of an agar-caustic mixture at the same *p*H. No correlation between colour and the presence of essential compounds has been found however. A proportion of the value of phosphate in the medium is due to its apparent combination with some constituent of agar, while the rest of its value probably lies in its buffering action.

It is not possible at the moment to assess the relative importance of the two effects. It seems as if together they account for the marked improvement the medium shows over the media previously used for the growth of lactic acid bacteria. Further, it is not possible to estimate whether some component in agar promotes growth of lactic acid bacteria in the absence of phosphate; indeed, since meat extract contains phosphate, it is unusual to prepare a medium in which agar has not been heated in a phosphate solution, generally, however, a much weaker solution than that used in the phosphate medium.

DISCUSSION

Owing to lack of knowledge of the precise food requirements for different bacteria, especially those which appear to need specific growth-promoting substances, the composition of media has up to the present time shown wide variation from laboratory to laboratory. Synthetic media for the growth of streptococci are gradually being defined(13), but these are obviously too complicated at present for routine use. The results given in the present paper were obtained during an attempt to rationalize the composition of a medium used primarily for the growth and isolation of lactic streptococci. Although the final recipe selected still contains substances such as peptone and meat extract which are liable to variation from batch to batch, the possible variations are reduced to a practicable minimum for a routine medium in the present state of knowledge and the medium is simple, cheap and easily prepared. It gives better results with lactic streptococci than we have so far obtained on any other medium, and it gives good growth of many other organisms.

Two most interesting points emerged in the course of the work:

(1) When agar is heated in a phosphate solution some substance is formed which has a growth-promoting action on the lactic acid bacteria.

(2) This growth-promoting substance is largely removed from the medium by filtration through paper pulp.

The second point has important implications. It suggests that filtration of media through adsorbing filters should be avoided whenever possible, and that if unavoidable the precise conditions of filtration should be specified. For example, standard media have been proposed for the bacteriological grading of milk. For some the method of

filtration is not specified; for others the method is specified up to a certain point but not up to a point which in the light of the present work would be necessary to produce a uniform result. For instance, if a paper pulp filter is to be used, the volume of medium to be filtered through a given weight of paper and the approximate time of filtration should both be specified. This would at any rate ensure that any growth-promoting substance adsorbed would be adsorbed equally from each batch of medium. The matter may not be equally important with all media; the effect of filtration seems in fact to be most striking when phosphate is a constituent of the medium. But even in media containing no added phosphate there is evidence of adsorption and consequent decrease in growth-promoting power. Thus, particularly with standard media for specific purposes, the details of filtration procedure need to be more precisely defined than has been the practice hitherto. It would in any case be better if filtration could be entirely avoided, and in our experience an unfiltered medium, although it contains a precipitate, gives no difficulty in practical use in Petri dishes.

SUMMARY

A cheap, easily prepared and relatively uniform agar medium, containing beef extract, lactose, peptone, yeast extract and phosphate, is described. It gives excellent results with lactic streptococci and a good growth with many other organisms.

The role of phosphate and agar in the medium and the effect of filtration is discussed. It is suggested that the value of phosphate is due partly to its buffering effect and partly to its combination with some constituent of agar to form a substance which has a growth-promoting action on the lactic acid bacteria. This active substance is largely removed from the medium if filtration through paper pulp is practised.

The author is indebted to Dr H. R. Whitehead for helpful criticism and advice.

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335. MISTS CONTAINING HYPOCHLORITE AS GERMICIDES IN THE DESTRUCTION OF AIR-BORNE BACTERIOPHAGES ATTACKING LACTIC STREPTOCOCCI

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(With 3 Figures)

Introduction

Probably the most dangerous vector of bacteriophage contamination of starters at cheese factories is the air. Starter 'failure' in the vat is frequently due to the bulk or mother starters being contaminated by air-borne phage. Apart from other sources of infection, e.g. dairy utensils and droplets from running whey, etc., the air in the neighbourhood of many cheese factories is constantly being charged with phage by the addition of the mist which is emitted from the whey separator.

One way of combating phage infection in a cheese factory would be the killing of all the phage particles in the air; and whilst this would be impractical for the entire air-space of a factory it may perhaps be a possible method for rendering phage-free the air of the relatively smaller enclosed space of the bulk starter room and laboratory (where the mother and submother cultures are transferred). Hunter & Whitehead (1), in liquid *in vitro* experiments, have shown that of several disinfectants which they investigated hypochlorite was by far the most effective and convenient for killing phage, and they suggested that air-borne contamination by phage might be overcome 'by a spray treatment with solution containing active chlorine'. Since this suggestion, it has become the routine practice at many cheese factories in this country (2,3) to spray the air with hypochlorite when phage attacks have caused frequent 'failure' of starters. The air has been sprayed in the laboratory before subculturing starters, and also in the bulk starter room before cooling the starter milk after 'sterilizing' it, and again before the inoculation of this milk for bulk starter. This spraying, although conducted in a haphazard manner at factories, appears to have been instrumental in the control of phage. Whitehead & Hunter, however, do not seem to favour the use of hypochlorite sprays, and more recently (4), in reporting investigations with hypochlorite as an aerial mist, state that 'the system of spray disinfection is actually being practised in at least two factories with a measure of success, but the idea of its general adoption had been abandoned in the meantime, although it may be applied in modified form in the future'.

It was thought that if phage could be sprayed artificially into a small room of dimensions such as might be utilized for a starter laboratory or a bulk starter room at a factory, and the air treated with a spray of hypochlorite, useful information might be obtained on the effectiveness of hypochlorite as an aerial disinfectant for the destruction of phage.

* Latterly with Deosan and Milton Antiseptic Limited

Experimental

I. *Phages selected*

The phage races studied, namely 144*f*, *y*1 and 236/9, were unrelated to each other; thus each phage reacted only with its own corresponding strain and did not cross-phage with either of the other two strains (5). The phages were prepared in yeast-dextrose broth (and for a few tests in whey, tests 5–15, Tables 9 and 10) and were selected from a collection of phages (5) which lysed *Str. cremoris* strains isolated from starter. The phages were selected on the basis of their ability to (i) produce a reasonably high concentration (of, at least, 10^8 particles per ml.) when grown with the host strain, (ii) form large and distinct plaques on yeast-dextrose agar, (iii) give rise only very slowly to secondary phage-resistant strains. Phage 144*f* fulfilled these three conditions and was used for the preliminary experiments and for the majority of the tests with disinfectants. Phages *y*1 and 236/9 were used to give additional comparative data on the effect of the disinfectants (with those obtained on phage 144*f*). The plaques formed by *y*1 and 236/9 on agar plates were small and sometimes the count was rendered unreliable by the uneven formation of plaques over the whole surface of the agar smear. The plaques of these two phages were often most distinct around the periphery of the smear, but obviously, since the whole plate was exposed uniformly to the phage-infected air, many plaques in the centre of the agar smear were missed in counting, owing to their indistinctness or invisibility. It seemed that this uneven formation of plaques was mainly due to the balance between the rate of growth of the host strain and the phage, for when the quantity of inoculum of strain which was smeared on the agar surface was reduced, there was an improvement in the uniformity of plaque formation of *y*1 over the whole agar smear. The inoculum usually consisted of approx. $\frac{1}{10}$ ml. of an 18 hr. broth culture taken from the deposit at the bottom of the broth. Lighter inocula consisted of approx. $\frac{1}{20}$ ml. only. The culture was smeared over the surface of agar, contained in Petri dishes, which had previously been warmed and dried in the 37° C. incubator, by using the short arm of an L-shaped glass rod. Adjusting the size of the inoculum did not improve the regularity of plaque formation for phage 236/9.

The strength of the phage preparation used was estimated by two different methods, using serial dilutions of the broth containing the phage, as detailed in an earlier paper (6). The tests employed consisted of a milk activity test depending on acid development and a broth test in which phaging was indicated by clearing of the turbidity.

The concentration of phage 236/9 was estimated in broth only, since the strain grew too slowly in milk for the activity test. The strengths of phages 144*f* and *y*1 were usually tested in broth only, since by either test these particular phage races gave similar results. Previously, it had been found that the activity test sometimes gave a higher reading than the broth test for certain phages (7).

Although more than one preparation of each phage was made, only phage of the same preparation was used for each series of experiments. The concentration of the phage was tested before commencing, but additional estimations were made when the experiments with the same preparation were extended over several days. The Seitz filtered phage preparation was stored at 3° C.

II. *Method of spraying*

A Dynalysor* was used to spray both the phage and disinfectant into the air of a closed room (details of this room are given below). The mechanism of this spray has been described in detail by Masterman (8), and the normal rate of spraying was about 1 g. of liquid per min. The preparation is disseminated throughout the air as a fine mist by using compressed air. The outlet blast of air, containing the mist of liquid under test, is so baffled that the droplets are very small as they emerge. In the Dynalysor there is a bakelite box section into which the liquid to be sprayed is poured and from which it finally emerges as a mist. This box may be disconnected from the main spraying machine and can be weighed before and after the liquid is sprayed. The approximate weight of liquid sprayed in a given time may thus be obtained. Separate boxes were employed for spraying phage and for each of the different kinds of disinfectants. They were thoroughly washed and dried before commencing each test.

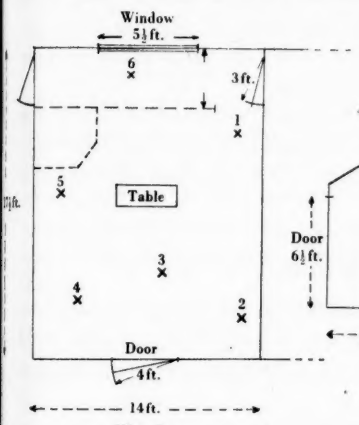

Fig. 1.

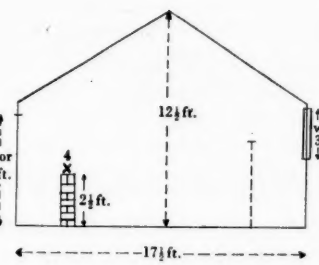

Fig. 2.

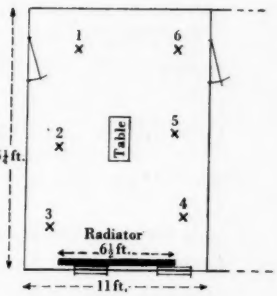

Fig. 3.

Fig. 1. Plan of room (bull-box) used as test chamber. × 1–6, positions at which agar plates were exposed.
Fig. 2. Elevation of room shown in Fig. 1. Fig. 3. Plan of room used for experiments at higher temperatures.

Phage was sprayed for periods of 10 min., except during a few of the preliminary tests. The disinfectants were also usually sprayed for periods of 10 min., but for some tests the spraying time was reduced to 5 min.

The strength of the hypochlorite solution and the concentration of the phage preparation, in some of the preliminary experiments, were tested before spraying, and the residues not used for spraying were retested again after spraying had ceased. A comparison of the two values obtained on the preparations before and after spraying showed no significant differences, and consequently it was considered that no 'distillation effect' of the air current had occurred during the process of spraying.

III. *Room used*

A newly built and previously unused bull-box was used as a test chamber into which the phages and disinfectants were sprayed.

Figs. 1 and 2 show the dimensions and general plan of the chamber. The cubic capacity

* Milton Antiseptic Limited, John Milton House, London, N. 7.

was approximately 2450 cu.ft. or 70,000 l. The room was built of the same kind of materials as many dairy premises. The floor consisted of cement, and the brick walls were faced with cement to a height of 3 ft. The roof was constructed from corrugated asbestos with reinforced concrete rafters. There were three doors and a window, but all openings were closed throughout the experimental periods. Between tests it was possible to flush the chamber with air quickly by opening all the doors and window, since, with the exception of one door leading into an adjacent room, all the openings communicated directly with the outside air.

During some of the preliminary tests, seven agar plates, previously smeared with the host strain, were exposed for each observation; otherwise agar plates were opened at six different stations in the room as shown in the plan. Tiers of bricks were erected so that the plates could be opened on the top of the brick platforms at $2\frac{1}{2}$ ft. above floor level. The agar plates were exposed to the air for 5, 10 or 15 min. in the preliminary tests, but for all the tests with disinfectant mists the time of exposure was standardized to 5 min. After exposing the plates, they were incubated at 30° C. and examined for plaques 18–24 hr. later. The plaques on all the plates, used at each exposure, were counted, and the average number of plaques per plate is given in the subsequent tables.

The Dynalysor was placed in the centre of the room on a small table and the spray was emitted $3\frac{3}{4}$ ft. above floor level. The slit sampler of Bourdillon, Lidwell & Thomas (9) (referred to later) was also placed on this table so that the air was sucked through into the slit from about $4\frac{1}{4}$ ft. above floor level.

IV. *Temperature and humidity control*

It was impossible to raise or lower at will the temperature of the room, and all tests, therefore, had to be conducted at the prevailing temperature, 30–50° F. (Tables 8–13 and 15), while normal temperatures in cheese rooms or starter rooms would be considerably higher.

The relative humidity during each test was determined from the wet and dry bulb thermometers suspended in the room.* With the exception of test 20 (Table 11), when the relative humidity was 71 %, the other humidities were higher, being at least 84 %.

The relative humidity of the air has been shown to be a factor of importance when the germicidal action of disinfectant mists is in question. Challinor (10) has summarized the findings of previous workers and points out that at temperatures between 54 and 74° F., with a relative humidity lower than about 71 %, a mist obtained from spraying a solution of hypochlorite into the air was not effective against bacteria. Elford & van den Ende (11) have also shown more recently that at 18–22° C. the relative humidity is an outstanding factor, and that below a relative humidity of 50 % bacterial destruction was negligible or slight.

Room used for experiments at higher temperatures. It was eventually possible to obtain another room in which some control over the relative humidity and temperature of the atmosphere could be exercised. This second room was similar in general construction and ventilation to the room used for all the other experiments, but it was unfortunately much higher (15 ft. as compared with 8 ft.), although the cubic capacity was nearly the same,

* It is realized that since the relative humidity values are based on readings taken in still air, they are slightly high.

i.e. 72,000 l. as compared with 70,000 l. (see Fig. 3). A radiator, 6½ ft. long, placed near the outside wall, was used to heat the air, and the temperature averaged 80° F. Calcium chloride contained in trays was placed on the floor overnight, and this, with the heat of the radiator, was sufficient to lower the relative humidity of the atmosphere to between 40 and 60 %. In order to increase the relative humidity to 70–90 %, steam was passed into the room for 5–10 min., and surplus water remained on the floor throughout the experiment to ensure continuous evaporation. A period of time was allowed to elapse so that the humidity could reach equilibrium before each experiment. Several readings were taken during each test, and the figures given in Tables 8–15 represent the mean relative humidity values computed from these readings.

METHODS OF RECOVERY OF PHAGE

I. *By 'natural' deposition (sedimentation)*

(*a*) *On to exposed surface of agar plates*

At the outset it was hoped to recover the phage, after it had been sprayed into the air of the room, by 'natural' deposition, i.e. settling on to yeast-dextrose agar plates, previously smeared with the host strain and exposed for varying time intervals. No guide could be found in the literature as to the suitability of this method, nor were any data available as to the persistency of phage particles in the atmosphere after they had been artificially sprayed into the air. Whitehead & Hunter (12) were able to demonstrate phage in factory environments by using agar plates and allowing the air to settle on the agar surface.

The cubic capacity of the room, the strength and the quantity of phage sprayed were all known factors, and thus the approximate number of phage particles sprayed into the room could be assessed. The number of plaques on the agar smear was taken to represent the number of phage particles recovered during the exposure of the plate. By assuming that Petri's formula quoted by Challinor (10) (the organisms present in 10 l. of air are deposited on 100 sq.cm. during 5 min. exposure) also applied to particles of phage, it was possible to determine the percentage recovery.*

After spraying 10–12 million particles of phage, i.e. 10–12 ml. of a phage preparation, with a concentration of 10^6 particles per ml. in 10 min., it will be seen (Table 1*a*) that

* On examining Petri's original paper (Petri, R. J. (1888), *Z. Hyg. InfektKr.* **3**, 1) it appears (p. 133) that the number of bacteria deposited on 100 sq.cm. in 10 min. when multiplied by a factor of 3–5 is approximately equal to the number present in a cu.m. of air. Hence, from this, in 3–5 min. the organisms deposited are equivalent to those present in a volume of air of 100 l. and not as has usually been assumed 10 l. In Tables 1, 2, 3 and 6 Petri's formula, in the amended form, has been applied to the data.

It is realized that Petri's formula was originally based on dry air-borne bacteria and not, as in the tests reported, on wet particles disseminated by a spray, where the rate of settling may vary with the size of particles produced by the type of spray which is used. Since the same method of spraying was employed throughout, i.e. both for the controls as well as for the disinfectant tests, the calculated percentage of phage survival, based on the numbers of particles present in the control, will remain relatively unchanged (Tables 8–15). The use of Petri's formula, however, for the calculation of the survival of phage particles in the 'natural' deposition experiments (Tables 1, 2, 3 and 6) is open to criticism. The low percentage recoveries of phage particles by the deposition method are borne out by the data obtained from observations when the slit sampler was employed, even though the plates tend to collect only the larger particles by deposition, while the slit sampler will collect smaller particles as well.

recoveries of about 0·5 % or less were obtained in the first 5 min. after spraying ceased, and these diminished progressively during the subsequent 30 min. An hour after spraying ceased, no phage particles were recovered. The count of plaques per plate was within the range of 50–100 during the first 5–10 min. If spraying of a disinfectant for a period of 5–10 min. were to be introduced, after the phage spraying ceased, the recovery of few, if any, phage particles would be expected, and the concentration of phage on which the disinfectant might act was considered inadequate. Wells, commenting on the work of Phelps (13), has remarked that he recovered only 25–50 % of the atomized organisms by means of Wells's air centrifuge.

Table 1. *Recovery of phage* 144*f by deposition on agar plates*

Preliminary test no.	Time for which phage sprayed (min.)	Concentration of phage preparation per ml.	Approx. concentration of phage particles sprayed per 10^3 l.	Time after phage spraying ceased (min.)	Time for which plate exposed (min.)	Average no. of plaques per plate (approx.)	% recovery
			(*a*) Phage sprayed for 10 min.				
3	10	10^6	14×10^4	0	5	56	0·5
				0	10	93	0·32
				38	10	2	0·007
				98	15	0	0
4	10	10^6	17×10^4	0	10	119	0·34
				30	10	6	0·02
				60	15	0	0
			(*b*) Phage sprayed for 15 or 20 min.				
6	15	10^6	24×10^4	0	10	144	0·28
				20	10	113	0·22
9	15	10^6	21×10^4	0	5	16	0·07
				5	10	21	0·05
				15	10	15	0·04
				30	10	10	0·02
11	15	10^6	18×10^4	0	10	15	0·045
				10	10	2	0·006
				20	10	1	0·003
12	20	10^6	28×10^4	0	10	30	0·05
				10	10	27	0·05
				20	10	11	0·02
14	20	10^6	33×10^4	0	10	11	0·015
				10	10	5	0·008
				20	10	3	0·004

The figures given in Table 1*b* show that increasing the amount of phage sprayed by lengthening the time of spraying to 15 or 20 min. does not, as might be expected, materially increase the count of plaques obtained by the 'natural' deposition method of recovery on plates.

(*b*) *On exposed surface of basins*

It was thought possible that the recovery of the phage particles might be low owing to their gravitation and settling on to the floor of the room, since it will be remembered that the plates were exposed 2½ ft. above ground level which is only about 1 ft. lower than the height at which the spray operated. To test this hypothesis empty sterile enamel basins (approximate area of exposed surface 200 sq.cm.) were exposed on the floor for periods varying from 10 to 45 min. With the exception of test 5 the percentage recovery of phage particles, by rinsing the basins with sterile milk and subsequently testing the concentration of phage in the milk, was still less than 0·4 % (Table 2). The phage particles,

therefore, did not appear to settle and accumulate on the floor of the room. Indeed, test 7, as well as two other preliminary tests not quoted in Table 2, indicated that rinsing with ¼ strength Ringer's solution failed to recover any particles.

(c) *On milk exposed in plates*

The recovery of phage particles from 10 ml. of sterile separated milk exposed in Petri dishes was still poor—less than 0·2 % (Table 3). The number of phage particles recovered in the milk during exposure was estimated by transferring the milk from the plates to sterile bottles and then applying the milk activity and broth tests (6). Where more than

Table 2. *Recovery of phage* 144*f by deposition on surface of basins exposed on the floor*

Preliminary test no.	Time for which phage sprayed (min.)	Concentration of phage preparation per ml.	Approx. concentration of phage particles sprayed per 10^3 l.	Time after phage spraying ceased (min.)	Time for which exposed (min.)	Area of basin (sq.cm.)	No. of particles per 100 sq.cm.	Medium with which basin rinsed	% recovery
5	20	10^6	28×10^4	0	30	189	5290	Milk	3·2
6	15	10^6	24×10^4	20	30	189	529	Milk	0·36
7	10	10^6	15×10^4	0	11	189	0	¼ Ringer's	0
				0	30	205	0	¼ Ringer's	0
10	20	10^6	27×10^4	0	45	189	529	Milk	0·32
				15	30	205	49	Milk	0·03

Table 3. *Recovery of phage* 144*f by deposition on* 10 *ml. of milk contained in Petri dishes*

Preliminary test no.	Time for which phage sprayed (min.)	Concentration of phage preparation per ml.	Approx. concentration of phage particles sprayed per 10^3 l.	Time after phage spraying ceased (min.)	Time for which exposed (min.)	No. of phage particles per 10 ml. of milk	% recovery
9	15	10^6	21×10^4	0	5	0	0
10	20	10^6	27×10^4	0	10	100	0·18
				10	10	10	0·018
				20	10	2	0·036
12	20	10^6	27×10^4	3	10	100	0·18
				13	10	10	0·018
				20	10	100	0·18
13	20	10^6	26×10^4	0	10	100	0·19
				10	10	2	0·004
				20	10	2	0·004

one plate containing milk was exposed at any one observation, the milk samples were mixed and the phage strength estimated on the bulk sample. Whitehead & Hunter (4) advocated this method for the recovery of phage at cheese factories in preference to exposing agar plates or aspirating phage-infected air through a liquid medium. This method, too, appears insufficiently sensitive to yield an adequate *quantitative* recovery of the phage sprayed.

II. *By aspiration of air*

(a) *Impingement on agar plates through slit sampler*

The value of the slit sampler recommended by Bourdillon *et al.* (9) for the recovery of phage was examined. In the slit sampler, air, sucked through at a rate of 1 cu.ft. or 28·6 l. per min., impinges on the surface of an agar plate (previously smeared with the

host strain). It will be seen from Table 4 that the spraying of preparations containing 10^6 particles of phage per ml. for 10 or even 20 min. still gives low and inadequate recoveries. Test 15 in Table 4 shows, however, that a preparation with a concentration of 10^7 per ml. gives 680 plaques even 30 min. after the spraying of the phage had ceased. This increase in the strength of phage, however, makes it impossible to determine the percentage destruction or recovery of phage particles from the air by agar plates for some time after the spraying of phage has ceased, since the large numbers of particles settling on the agar produce innumerable plaques or even lyse the whole of the culture.

Table 4. *Recovery of phage* 144*f by aspiration of air through slit sampler on to agar plates after* 1 *min. exposures. Volume of air drawn through* = 1 *cu.ft.* (28·6 *l.*)

Preliminary test no.	Time for which phage sprayed (min.)	Concentration of phage preparation per ml.	Approx. concentration of phage particles sprayed per 10^3 l.	Time after phage spraying ceased (min.)	No. of plaques per plate (approx.)	% recovery
7	10	10^6	15×10^4	8	9	0·2
				30	0	0
10	20	10^6	27×10^4	0	64	0·8
				10	14	0·2
				30	3	0·04
11	10	10^6	18×10^4	0	26	0·5
				10	10	0·2
				30	0	0
13	15	10^6	26×10^4	0	140	1·9
				10	18	0·2
				30	10	0·1
15	10	10^7	16×10^4	0	∞	—
				10	∞	—
				30	680	1·5

Table 5. *Recovery of phage* 144*f by aspiration of air through broth*

Preliminary test no.	Time for which phage sprayed (min.)	Concentration of phage preparation per ml.	Approx. concentration of phage particles sprayed per 10^3 l.	Time after phage spraying ceased (min.)	Vol. of air drawn through	No. of phage particles recovered per 150 ml. broth	% recovery
14	20	10^6	33×10^4	12	4 l. in 1½ min.	150	11·4
15	10	10^7	16×10^5	0	4 l. in 2½ min.	1,500	23·9
16	10	10^7	14×10^5	0	4 l. in 3½ min.	1,500	27·6
				15	4 l. in 3½ min.	1,500	27·6
17	10	10^7	14×10^5	0	4 l. in 3½ min.	1,500	2·6
				15	4 l. in 3½ min.	1,500	2·6
18	15	10^8	2×10^7	0	4 l. in 3½ min.	15,000	18·6
				14	4 l. in 3½ min.	150	0·19
22	10	10^{12}	13×10^{10}	0	2½ l. in 7 min.	100,000	0·03
				10	2½ l. in 7 min.	100,000	0·03

(*b*) *Aspiration through broth medium*

As the use of the slit sampler also seemed to have disadvantages for estimating the recovery of phage, preliminary tests were made in which the phage-infected air was aspirated through yeast-dextrose broth. The number of phage particles recovered was then estimated by serial dilutions of the broth. The data from Table 5 show that with a reasonably strong phage this could be a suitable method of recovery. In practice the broth frothed, and when the air was aspirated through broth containing glass beads or the collecting liquid was changed to milk, frothing was still excessive. From Table 5 it will also be seen that the rate of air aspiration was not uniform, and considerable

variations occurred within the different tests. The difficulties due to frothing did not recommend this method for further trial, but if it had been used extensively it would have been necessary to standardize the speed of air aspiration.

(c) *Impingement on gelatine plates through slit sampler*

The last two methods of recovery of phage, viz. the estimation of plaques on agar using the slit sampler and the evaluation of phage strength in a liquid by drawing air through broth, have limitations. It was thought that if the air through the slit sampler could be drawn on to a liquid medium, contained in a Petri dish, frothing might be avoided. At the same time a method of estimation of the phage strength by serial dilution for all the readings in each test might still be applied. It was found, when air was drawn in at the normal rate, so that it impinged on the liquid in a plate, that a depression formed in the liquid. There were also the attendant dangers of splashing or scattering the liquid and perhaps of the phage particles rebounding from the exposed glass surface of the plate after impact. Bourdillon *et al.* (9) have suggested that solid media (agar) on the Petri dish might be replaced by a non-volatile harmless liquid which would be sufficiently viscous to avoid being scattered by the air stream. By this method, after drawing the air through on to the liquid, the phage strength could be estimated by dilution of the 'collecting' liquid. Medicinal paraffin was suggested by Bourdillon *et al.* (9) for the 'collecting' liquid, but as they state, it is messy and hence not to be recommended.

Sodium lactate, sodium silicate and glycerine were all used as liquids for recovering phage from the air by the slit-sampler equipment, but these liquids were not sufficiently viscous to overcome scattering by the air stream. The use of a semi-solid medium—dilutions of agar ranging from 0·05 to 0·5 %—was then investigated. These agar preparations were not successful, since they were insufficiently viscous, could not be liquefied at room temperatures, and consequently would not give a uniform liquid from which to estimate the phage strength.

Finally, tests with gelatine, instead of agar, showed that it could be used as a solid medium on which phage particles of uncountable numbers could be collected and yet the medium could be easily and quickly liquefied at a low temperature and the concentration of phage estimated per 10 ml. of the liquid gelatine by the broth test. In order to reduce the gelatine content in the dilutions for the broth tests the original collecting medium consisted of 5 % cleared gelatine in nutrient broth (0·15 % yeastrel, 0·5 % lemco, 0·5 % peptone, 0·25 % salt, *p*H 7). This gelatine medium was sterilized in the steamer at 100° C. for 30 min. on 3 successive days. Before use in the sampler it was measured into Petri dishes in 10 ml. quantities, and after solidifying it was 'hardened' in the refrigerator overnight.

As mentioned previously, a high level of phage concentration was necessary in the test room so that the experiments with disinfectants could be applied to a wide range of phage concentration. When smeared agar plates were exposed after spraying phage of high concentration, innumerable plaques were always obtained, although after spraying the disinfectant, the number of plaques was usually very low. No calculation could, therefore, be made of the percentage recovery or of the relative efficiency of the disinfectant. When the gelatine plates were used in the slit sampler, estimations of the phage strength by dilution of the gelatine allowed such a computation to be made.

The number of phage particles recovered by exposing gelatine plates in the slit sampler 30 min. after spraying ceased (Table 6) shows that provided a greater concentration than 10^6 per ml. was used, sufficient phage particles were present to investigate the action of disinfectant mists. The percentage recoveries of phage particles, calculated by Petri's formula, from the plaque counts, obtained by the 'natural' deposition of phage on exposed agar plates, were comparable with those figures obtained by the method of artificially sucking phage-infected air on to gelatine. Thus, even if the assumption of Petri's formula for bacteria may not hold for phage, it would appear that in order to study the effect of a disinfectant either method would give comparable results (see footnote, p. 295).

Table 6. *Recovery of phage* 144*f by aspiration of air through slit sampler in* 3 *min. exposures on to* 5 % *gelatine plate* (*volume of air drawn through* = 3 *cu.ft.* (85·8 *l.*)) *and a comparison with that obtained by 'natural' deposition on agar plates*

Preliminary test no.	Time for which phage sprayed (min.)	Concentration of phage preparation per ml.	Approx. concentration of phage particles sprayed per 10^3 l.	Time after phage spraying ceased (min.)	No. of phage particles recovered per gelatine plate by aspiration	% recovery	Average no. of plaques per agar plate 10 min. exposure (approx.) by deposition	% recovery
17	10	10^8	14×10^6	2	20,000	4·8	—	—
				12	2,000	0·4	665	0·23
				30	200	0·02	94	0·03
19	15	10^8	2×10^7	0	20,000	3·4	—	—
				11	2,000	0·34	—	—
				35	200	0·01	102	0·003
20	10	10^8	13×10^6	0	1,000,000	90	—	—
				12	100,000	9	—	—
				32	1,000	0·09	766	0·03

Experiments with hypochlorite mists

In all the following experiments in which the germicidal effect of disinfectant mists on phage has been investigated, three methods of recovery of phage from the atmosphere were finally used which made it possible to estimate the concentration of phage particles in the air over a wide range.

(1) 'Natural' deposition of phage particles from the air on to agar plates, previously smeared with the host strain, during 5 min. exposures of the plates at different time intervals after the spraying of the phage had ceased.

(2) Aspiration of air containing phage for 1 min. through the slit sampler and impingement on to agar plates previously smeared with the host strain. The volume of air impinged on to the plate in this time is 1 cu.ft. (28·6 l.). The number of plaques obtained from the agar plate used at each different exposure is given.

(3) Aspiration of air containing phage for 3 min. through the slit sampler and impingement on to gelatine plates. The volume of air drawn through the slit on to the plate is 3 cu.ft. (85·8 l.). In the tables, the phage concentration, demonstrated by the broth test, is recorded per ml. of gelatine.

To facilitate calculation, the concentration of hypochlorite in the mists has been given throughout as p.p.m. av./cl., although the effective germicidal agent in air is hypochlorous acid gas. For example, in Table 9, test 8, the concentration of hypochlorite mist, computed from the weight of hypochlorite sprayed, is given as 0·014 p.p.m. av./cl. which is equivalent by volume to 4·2 p.p.m. hypochlorous acid gas.

I. *Control tests—effect of salt and water*

In order to test the efficiency of hypochlorite as a disinfectant for air-borne phage, the effect of commercial hypochlorite solutions of varying strength was examined. The concentrations of the solutions used are given below. Many of the hypochlorite solutions available commercially are proprietary brands and they contain common salt in addition to hypochlorite. For convenience, three solutions (Table 7) already available for the dairy trade, or their dilutions, were used. Four different samples of solution 3 were examined. The salt concentration of these solutions and the *p*H values are also given. An undiluted hypochlorite solution as 'approved' by the Ministry of Agriculture and Fisheries for dairy purposes contains 9–12 % av./cl.

Table 7

Hypochlorite solution	Approx. % av./cl.*	Approx. % salt	*p*H†
Diluted 1	0·1	1·6	10·84
1	1·1	16	11·82
Diluted 2	5·0	5	12·55
3	8·8	15	12·84
2	10·0	10	12·83
3	11·4	15	12·95
3	13·0	15	12·95
3	13·3	15	12·95

* av./cl. = available chlorine.
† *p*H determined with the 'Alki' glass electrode (Cambridge Instrument Co.).

When the germicidal effect of hypochlorite mists was examined, *N*/1000 sodium thiosulphate was added, immediately prior to pouring, to all the gelatine and yeast-dextrose agar plates which were used in the experiments. The thiosulphate was added to nullify any inhibitory effect which the hypochlorite might have had on the phage after it had been deposited on the plate. Previous investigation had shown that with this strength of thiosulphate there was no perceptible reduction in the size or the number of plaques formed.

When the action of hypochlorites, used as disinfectant mists, was explored, the control tests consisted of spraying phage for 10 min. For the actual test with hypochlorite a similar spraying of phage occurred, after which the test hypochlorite solution was sprayed. However, additional controls to examine the effect of spraying distilled water alone or 15 % salt solution for 10 min. instead of hypochlorite solution were also made, since it was not known whether the spraying of such liquids would aid mechanical removal of phage from the air or even actually destroy it. The increase in humidity due to the 10 ml. of water would be very slight. In Table 8 a comparison is made between test 38, when phage alone was sprayed, and test 39, when distilled water was sprayed after the phage. The results indicate that the spraying of distilled water did not wash down or remove any phage particles. The results of test 40 suggest that the salt solution might cause a reduction in recovery, but this is not borne out by those from tests 45 and 46 in which no deleterious effect can be ascribed to the salt solution. The results of both the exposed agar plates and the gelatine plates in these experiments show remarkable agreement.

When phage *y* 1 was examined (tests 41 and 42, Table 12) there was again no perceptible deleterious effect on the phage after spraying 15 % salt solution, but results from tests 31 and 33 suggest that distilled water only had a slight effect on the survival of *y* 1. However, with phage 236/9 (tests 27 and 37, Table 13) the spraying of water did not produce any change. Incidentally, since in practice commercial hypochlorites contain salt, the action of saline can hardly be separated from that of hypochlorite and probably a stricter control for the effect of hypochlorite solutions would be exercised by the spraying of water alone.

Table 8. *The effect of water and* 15 % *salt solution on the recovery of phage* 144*f*

Test no. ...		38	39	40	46	45
		Control, phage alone	Distilled water	15 % salt solution	Control, phage alone	15 % salt solution
Concentration of phage preparation per ml.		10^9	10^9	10^9	10^{10}	10^{10}
Approx. concentration of phage particles sprayed per 10^3 l.		14×10^8	11×10^8	11×10^8	12×10^8	7×10^8
Method of recovery	Time after phage spraying ceased (min.)	Average no. of plaques per plate				
(1) Sedimentation on agar plates	10	∞	∞	420	∞	∞ ∞
	20	671	580	53	∞	∞
	40	25	57	2	983	1890
	55	6	4	0	686	490
	65	0	0	1	405	255
		No. of plaques per plate				
(2) Aspiration through slit sampler on to agar plates	14	∞	∞	540	∞ ∞	.
	30	∞	∞	35	∞ ∞	∞ ∞
	50	130	230	0	∞	∞ ∞
		Concentration of phage per ml. gelatine				
(3) Aspiration through slit sampler on to gelatine plates	0	10^4	—	—	10^5	—
	10	10^4	10^4	10^2	10^4	.
	16	10^4	10^4	10^2	10^4	10^4
	24	10^3	10^3	10^1	10^4	10^4
	35	10^3	10^3	10^1	10^4	10^5
	45	10^3	10^2	10^1	10^4	10^3
	60	10^1	10^1	****	10^3	10^3
Temp. of room ° F.		42	44	37	40	40
Relative humidity		86	87	94	86	86

Key: . = no test. **** = not found in 1 ml.

At higher temperatures, too (77–79° F.), a comparison of tests 59 and 58 (Table 14) shows that the spraying of 10 g. of water had no effect in reducing the efficiency of phage recovery, and further, the actual amount of water sprayed did not increase the apparent humidity of the room to any significant extent.

II. *Hypochlorite solutions sprayed immediately after spraying of phage* 144*f had ceased*

Experiments in which hypochlorite solutions of 0·1, 1·1, 8·8 and 10 % av./cl. were sprayed for 10 min. after the phage had been atomized into the atmosphere, also for 10 min., are summarized in Table 9. The phage concentration estimated both from the gelatine plate method and the counts of plaques on agar plates show that by spraying

a solution containing 0·1 % av./cl. less than 1 % of the phage particles survived 10 min. after phage spraying ceased. In this mist (test 7) the concentration p.p.m. of av./cl. was very low, being only 0·00014. A similar destruction was also demonstrated after the spraying of stronger solutions. Since solutions of 0·1 % av./cl., when sprayed for 10 min., proved germicidal in the room used, it was decided to determine whether smaller concentrations of hypochlorite, obtained by reducing the interval of spraying, would suffice. The results given in Table 10 show that reducing the spraying time of hypochlorite (0·1 % av./cl.) solution from 10 to 5 min., i.e. so that the initial concentration, p.p.m. of av./cl. in the room used was reduced to 0·00009, did not produce a sufficiently germicidal effect, the survival of particles of phage after 10 min. exposure being about 10 %. When the solution sprayed contained 1·1 % av./cl. the destruction was again 99 % after spraying for 5 min. only. Hence mists obtained after spraying solutions of 1–10 % av./cl. for 10 min., i.e. giving initial concentrations of av./cl. in the air of 0·0014–0·014 p.p.m. immediately after spraying of phage had ceased, appear to be sufficiently germicidal to give a margin of safety when an atmosphere is to be freed of air-borne phage.

III. *Persistency of germicidal action of hypochlorite mists after spraying*

In cheese-making practice, the recontamination of the atmosphere with phage may be almost a continuous process and, although the atmosphere might be free from phage at any one time after spraying with hypochlorite solution, it remains to determine for how long such a mist might be effective. In tests 16–22 (Table 11) hypochlorite solution was sprayed first, and when spraying had ceased, phage was then sprayed into the atmosphere.

The germicidal action of the mist (0·0006 p.p.m. av./cl.) from a 1·1 % hypochlorite solution did not persist long enough to exert a sufficiently strong effect on phage introduced into the atmosphere after hypochlorite had been sprayed (test 17). When stronger solutions containing 5 or 10 % av./cl. were sprayed, a satisfactory result was obtained, although there was an apparent 10 % survival of phage particles immediately after the spraying of phage had ceased. This is perhaps to be expected, since there would be a time interval before the majority of the phage particles had been attacked by the hypochlorite mist, even if, finally, the disinfectant was sufficiently potent to destroy all the phage particles present. Ten minutes after phage spraying ceased, the process of phage destruction was almost complete. In tests 21 and 22, a 10 min. interval was allowed to elapse after spraying hypochlorite solutions of 10 and 13·3 % av./cl. respectively before spraying the phage preparation. It will be seen that the mists, which when first sprayed contained 0·014 and 0·02 p.p.m. av./cl. respectively, were sufficiently persistent to produce 99 % destruction of phage particles 16 min. after the spraying of phage had ceased.

IV. *Supplementary tests with phages y*1 *and* 236/9

The results of tests 35 and 32 (Table 12) show that spraying hypochlorite solutions of 1·1 and 10 % av./cl. for 10 min. were both equally effective in the complete destruction of phage *y*1. When a 10 % av./cl. hypochlorite solution (test 34) was sprayed into the air before phage *y*1 was introduced, only 1·0 % of the particles survived 16 min. after phage spraying had ceased.

Table 9. *The germicidal effect on phage of spraying hypochlorite solutions into the air immediately after the spraying of phage 144f had ceased*

Method of recovery	Test no. ...	5	7	6	8	9	10	11
	Concentration of hypochlorite solution (% av./cl.)	Control	0·1	1·1	10	Control	1·1	8·8
	Approx. concentration of hypochlorite mist (p.p.m. av./cl.)	—	0·00014	0·0014	0·014	—	0·0013	0·009
	Concentration of phage preparation per ml.	10^{12}	10^{12}	10^{12}	10^{12}	10^{12}	10^{12}	10^{12}
	Approx. concentration of phage particles sprayed per 10^6 l.	12×10^{10}	11×10^{10}	14×10^{10}	14×10^{10}	16×10^{10}	12×10^{10}	11×10^{10}
	Time after phage spraying ceased (min.)	Average no. of plaques per plate						
(1) Sedimentation on agar plates	0	∞ ∞	—	—	—	.	—	—
	10	∞ ∞	21	1	0	∞	1	0
	20	∞ ∞	12	10	0	∞	3	0
	40	∞ ∞	0	8	0	1600	6	0·4
	55	∞ ∞	1	5	0	548	2	0·4
	65	∞ ∞	1	11	1	235	2	0·8
	75	∞ ∞	1	11	0	104	1	1·0
	85	∞ ∞	1	9	0	.	.	—
		No. of plaques per plate						
			% survival		% survival		% survival		% survival		% survival		% survival
(2) Aspiration through slit sampler on to agar plates	14	∞ ∞	0	2	0	0	∞	0	0	2	0
	30	∞ ∞	3	19	0	0	∞	2	0	0	4	—
	50	∞ ∞	2	12	0	0	∞	0	—	0	4	—
	70	∞ ∞	3	6	0	0	1200	1	0·08	0	0	—
	80	∞ ∞	8	2	0	0	.	.	—	.	.	—
		Concentration of phage per ml. of gelatine											
			% survival		% survival		% survival		% survival		% survival		% survival
(3) Aspiration through slit sampler on to gelatine plates	0	10^6	—	—	—	10^6	—	10^6	—	—	—	—
	10	10^6	10^5	0·001	10^2	0·01	10^4	0·001	10^5	***	< 0·01	***	< 0·01
	16	10^5	10^5	0·01	10^4	0·01	10^4	0·01	10^5	***	< 1·0	10^5	1·0
	24	10^5	10^5	0·01	10^3	0·1	10^3	0·01	10^5	***	1·0	10^5	1·0
	35	10^4	***	< 0·1	10^4	0·1	***	< 0·01	10^5	10^5	1·0	***	1·0
	45	10^4	***	< 0·1	10^4	0·1	***	< 0·1	10^4	***	< 0·1	***	0·1
	60	10^3	***	< 1·0	10^4	1·0	***	< 1·0	10^5	***	< 10·0	***	< 10·0
	75	10^4	***	< 0·1	***	0·1	***	< 0·1	10^5	***	< 10·0	***	< 10·0
	85	10^5	***	< 0·01	10^5	0·01	***	< 0·01	.	.	—	.	—
	Temp. of room, °F.	32		39		32		39		33		35		36	
	Relative humidity			85				86		84		84		85	

Key: · =no test. *** =not found in 10^1.

From the data of Table 13 (tests 25, 26) phage 236/9 appears to be almost completely destroyed by mists obtained by spraying hypochlorite solutions of 1·1 and 10 % av./cl., and in a hypochlorite mist sprayed immediately before phage 236/9 (test 29) the particles did not survive appreciably 10 min. after the phage spraying had ceased. When

Table 10. *The germicidal effect on phage of spraying hypochlorite solutions of* 0·1 *and* 1·1 % *av./cl. into the air for* 5 *min. immediately after the spraying of phage* 144*f had ceased*

	Test no. ...	12	15		13	
Concentration of hypochlorite solution (% av./cl.)		Control	0·1		1·1	
Approx. concentration of hypochlorite mist (p.p.m. av./cl.)		—	0·00009		0·0005	
Concentration of phage preparation per ml.		10^{12}	10^{12}		10^{12}	
Approx. concentration of phage particles sprayed per 10^3 l.		14×10^{10}	12×10^{10}		11×10^{10}	
Method of recovery	Time after phage spraying ceased (min.)	Average no. of plaques per plate		% survival		% survival
(1) Sedimentation on agar plates	10	∞	∞	—	11	—
	20	∞	∞	—	6	—
	40	2155	281	13·0	4	0·2
	55	1331	128	9·8	3	0·5
	65	615	41	6·6	2	0·3
	75	478	25	5·2	3	0·6
		No. of plaques per plate				
(2) Aspiration through slit sampler on to agar plates	14	∞	∞	—	3	—
	30	∞	560	—	3	—
	50	∞	240	—	1	—
	70	884	75	8·4	0	0
		Concentration of phage per ml. gelatine				
(3) Aspiration through slit sampler on to gelatine plates	0	10^6	.	—	.	—
	5	.	10^3	0·1	10^1	0·001
	10	10^4	10^3	10·0	10^2	1·0
	16	10^5	10^2	0·1	10^1	0·01
	24	10^5	10^2	0·1	***	<0·01
	35	10^2	10^2	10·0	10^1	1·0
	45	10^4	10^1	0·1	10^1	0·1
	60	10^3	10^1	1·0	***	<1·0
	75	10^3	10^2	10·0	.	—
Temp. of room, ° F.		34	35		35	
Relative humidity		85	85		85	

Key: · = no test. *** = not found in 10^1.

a 10 min. interval elapsed between the end of spraying a mist from a 13·0 % av./cl. solution of hypochlorite (test 30) and the commencement of spraying phage 236/9, the survival of phage particles 10 min. after the spraying of phage had ceased was also low. These results with phages *y*1 and 236/9 are very similar to those obtained when the germicidal action of hypochlorite solutions on phage 144*f* was examined.

Table 11. *The germicidal effect on phage of spraying hypochlorite solutions into the air for 5 or 10 min. before spraying phage 144f*

		Hypochlorite sprayed immediately before the phage				10 min. interval after hypochlorite sprayed before phage sprayed	
Test no. ...		16	17	19	18	20	21	22
Concentration of hypochlorite solution (% av./cl.)		Control	1·1	5	10	10	10	13·3
Hypochlorite spraying period (min.)		—	5	5	5	10	10	10
Approx. concentration of hypochlorite mist (p.p.m. av./cl.)		—	0·0006	0·0036	0·003	0·013	0·014	0·02
Concentration of phage preparation per ml.		10^{10}	10^{10}	10^{10}	10^{10}	10^{10}	10^{10}	10^{10}
Approx. concentration of phage particles sprayed per 10^3 l.		16×10^3	16×10^3	12×10^3	14×10^3	16×10^3	14×10^3	16×10^3
Method of recovery	Time after phage spraying ceased (min.)	Average no. of plaques per plate											
(1) Sedimentation on agar plates	10	∞ ∞ ∞	∞	—	727	—	∞	—	9	—	∞	—	1890	—
	20	∞ ∞ ∞	∞	—	56	—	275	—	5	—	560	—	626	—
	40	∞ ∞ ∞	∞	—	14	—	33	—	0	—	71	—	92	—
	55	1433	444	30·9	3	0·2	18	1·0	3	0·2	14	0·9	37	2·5
	65	789	125	15·8	2	0·2	4	0·5	0	0	7	0·7	8	1·0
	75	476	60	12·6	2	0·4	6	1·23	5	1·0	1	0·2	3	0·6
		No. of plaques per plate												
(2) Aspiration through slit sampler on to agar plates	14	∞ ∞ ∞	∞	—	1000	—	285	—	1	—	∞	—	∞	—
	30	∞ ∞ ∞	∞	—	130	—	53	—	4	—	200	—	680	—
	50	∞ ·	∞	—	7	—	13	—	0	0	35	—	133	—
	70	∞	35	—	0	0	4	—	1	—	3	—	23	—
		Concentration of phage per ml. gelatine												
(3) Aspiration through slit sampler on to gelatine plates	0	10^4	10^4	100	10^3	10·0	10^3	10·0	10^3	10·0	10^3	10·0	10^3	10·0
	10	10^4	10^4	100	10^3	10·0	10^2	1·0	10^1	0·1	10^3	10·0	10^3	10·0
	16	10^5	10^3	1·0	10^2	0·1	10^1	0·01	***	<0·01	10^2	0·1	10^3	1·0
	24	10^4	10^3	10·0	10^1	0·1	10^2	1·0	****	0	10^2	1·0	10^2	1·0
	35	10^4	10^3	10·0	10^1	0·1	10^1	0·1	.	—	10^2	1·0	10^2	1·0
	45	10^5	10^2	0·1	10^1	0·01	***	<0·01	Found in 1 ml.	0·001	10^1	0·01	10^2	0·1
	60	10^4	10^3	10·0	10^1	0·1	***	<0·1	****	0	***	<0·1	10^2	1·0
	75	10^3	10^1	1·0	10^1	1·0	***	<1·0	****	0	***	<1·0	****	0
Temp. of room, °F.		34	35		34		35		37		34		31	
Relative humidity		84	85		85		85		71		85		90	

Key: · =no test. *** =not found in 10^1. **** =not found in 1 ml.

Table 12. *The germicidal effect on phage of spraying hypochlorite solutions into the air before and after the spraying of phage y1 had ceased*

		Hypochlorite sprayed before phage
Test no. ...		23
Concentration of hypochlorite solution (% av./cl.)		10
Hypochlorite spraying period (min.)		10
Approx. concentration of hypochlorite mist (p.p.m. av./cl.)		0·014
Concentration of phage preparation per ml.		10^{10}
Approx. concentration of phage particles sprayed per 10^3 l.		14×10^3
Method of recovery	Time after phage spraying ceased (min.)	Average no. of plaques per plate
(1) Sedimentation on agar plates	10	1890
	20	626
	40	92
	55	37
	65	8
	75	3
		No. of plaques per plate
(2) Aspiration through slit sampler on to agar plates	14	∞
	30	680
	50	133
	70	23
		Concentration of phage per ml. gelatine
(3) Aspiration through slit sampler on to gelatine plates	0	10^3
	10	10^3
	16	10^3
	24	10^2
	35	10^2
	45	10^2
	60	10^2
	75	****
Temp. of room, °F.		31
Relative humidity		90

Key: **** =not found in 1 ml.

Table 12. *The germicidal effect on phage of spraying hypochlorite solutions into the air before and after the spraying of phage y1 had ceased*

					Hypochlorite sprayed immediately after phage				Hypochlorite sprayed before phage			
									Immediately		With 10 min. intervals after hypochlorite spraying had ceased	
Test no. ...		41	42	31	33	35		32	34		36	
Concentration of hypochlorite solution (% av./cl.)		Control	15% salt solution	Control	Distilled water	1·1		10	10		13	
Approx. concentration of hypochlorite mist (p.p.m. av./cl.)		—	—	—	—	0·0014		0·014	0·014		0·017	
Concentration of phage preparation per ml.		10^8	10^8	10^8	10^8	10^8		10^8	10^8		10^8	
Approx. concentration of phage particles sprayed per 10^3 l.		14×10^6	14×10^6	16×10^6	14×10^6	14×10^6		14×10^6	12×10^6		11×10^6	
Method of recovery	Time after phage spraying ceased (min.)	Average no. of plaques per plate										
							% survival		% survival		% survival	
(1) Sedimentation on agar plates	10	∞	∞	∞	728	2	—	0	0	700	—	921	—
	20	26	62	∞	47	0	0	0	0	126	—	111	—
	40	4	13	292	1	0	0	0	0	0	0	0	0
	55	1	1	12	0	·	—	0	0	·	—	0	0
	65	·	·	3	·	·	—	·	—	·	—	·	—
		No. of plaques per plate											
							% survival		% survival		% survival		% survival
2) Aspiration through slit sampler on to agar plates	14	∞	∞	∞	326	0	0	6	—	∞	—	2050	—
	30	17	760	∞	120	2	—	0	0	70	—	73	—
	50	·	·	∞	0	·	—	·	—	·	—	·	—
		Concentration of phage per ml. gelatine											
							% survival		% survival		% survival		% survival
(3) Aspiration through slit sampler on to gelatine plates	0	10^4	—	10^5	—	—	—	—	—	10^3	1·0	10^4	10·0
	10	10^3	10^3	10^4	10^4	****	0	****	0	10^4	100	10^4	100
	16	10^3	10^3	10^4	10^2	****	0	****	0	10^2	1·0	10^3	1·0
	24	10^2	10^2	10^4	10^2	****	0	****	0	10^3	1·0	10^3	10·0
	35	Found in 1 ml.	10^2	10^3	10^3	****	0	****	0	10^2	10·0	10^1	1·0
	45	10^1	10^1	10^3	Found in 1 ml.	****	0	****	0	Found in 1 ml.	0·1	10^1	1·0
	60	—	—	Found in 1 ml.	****	****	0	—	—	·	—	·	—
Temp. of room, °F.		48	48	49	48	48		49	48		40		
Relative humidity		88	88	96	88	88		89	88		86		

Key: · = no test. **** = not found in 1 ml.

Table 13. *The germicidal effect on phage of spraying hypochlorite solutions into the air before and after the spraying of phage* 236/9 *had ceased*

				Hypochlorite sprayed immediately after phage		Hypochlorite sprayed before phage				
						Immediately		With 10 min. interval after hypochlorite spraying had ceased		
Test no. ...		27	37	26	25	29		30		
Concentration of hypochlorite solution (% av./cl.)		Control	Distilled water	1·1	10	10		13		
Approx. concentration of hypochlorite mist (p.p.m. av./cl.)		—	—	0·0013	0·01	0·013		0·019		
Concentration of phage preparation per ml.		10^8	10^8	10^8	10^8	10^8		10^8		
Approx. concentration of phage particles sprayed per 10^3 l.		14×10^6	16×10^6	12×10^6	10×10^6	20×10^6		14×10^6		
Method of recovery	Time after phage spraying ceased (min.)	Average no. of plaques per plate								
					% survival		% survival		% survival		% survival
(1) Sedimentation on agar plates	10	∞	∞	0	0	0	0	∞	—	∞	—
	20	∞	812	3	—	0	0	0	0	39	—
	40	1	5	0	0	0	0	1	—	1	—
	55	0	0	0	0	0	0	0	0	5	0
	65	0	.	.	.	.	—	0	0	0	0
		No. of plaques per plate									
					% survival		% survival		% survival		% survival
(2) Aspiration through slit sampler on to agar plates	14	∞	∞	0	0	0	0	102	—	∞	—
	30	0	∞	2	0	0	0	20	0	0	0
	50	0	.	.	—	.	—	0	0	0	0
		Concentration of phage per ml. gelatine									
					% survival		% survival		% survival		% survival
(3) Aspiration through slit sampler on to gelatine plates	0	10^4	—	—	—	—	—	10^2	1·0	10^4	100
	10	10^4	10^4	***	<0·1	***	<0·1	10^1	0·1	10^2	1·0
	16	10^4	10^3	***	<0·1	***	<0·1	10^1	0·1	10^1	0·1
	24	10^3	10^3	***	<0·1	***	<1·0	10^1	1·0	10^1	1·0
	35	10^1	10^2	****	0	****	0	****	0	Found in 1 ml.	10
	45	Found in 1 ml.	10^2	****	0	****	0	****	0	****	0
	60	Found in 1 ml.	.	.	—	.	—	****	0	****	
Temp. of room, °F.		46	41	46		46		46			
Relative humidity		88	86	88		88		96		96	

Key: · = no test. *** = not found in 10^1. **** = not found in 1 ml.

V. *Effect of higher temperatures and variable humidities*

The results of the tests carried out at the higher temperatures (approx. 80° F.) with all three phages are summarized in Table 14. The effect of changes in relative humidity was also investigated at the same time.

The results of tests 49 and 50 show that at a relative humidity of 50 % there was a persistent survival of 10 % of the particles of phage 144*f* throughout the test. When the relative humidity was raised to 65 % (tests 51 and 52), destruction was complete in the first few minutes. With phages *y*1 and 236/9 a comparison of the results of tests 57 and 58 with tests 60 and 61, and of tests 62 and 63 with tests 64 and 65 shows that at a relative humidity of about 50 % none of the phage particles was destroyed in spite of the fact that a solution of 10 % av./cl. was used. On raising the humidity to about 75 %, phage destruction was complete.

From a comparison of the data of tests 50 and 52, 54 and 56, 58 and 61, 63 and 65 (Table 14), it appears that recovery of phage particles is enhanced at low relative humidities.

Above 80° F. air sucked through the slit sampler and impinging on the gelatine plates caused the liquefaction of the gelatine medium, especially during the tests at high relative humidity, and there was the possibility of some loss of liquid through splashing. The plates were stored in a refrigerator (38° F.) and were only removed immediately before exposure, but no other attempt was made to keep the plate cool during exposure in the slit sampler.

VI. *Tests with mists other than from hypochlorite solutions*

The literature on the spraying of aerial disinfectants shows that several compounds in the substituted phenol group alone or when dissolved in propylene glycol as well as propylene glycol itself are all effective germicides. A few experiments were performed to test the efficiency of some of these compounds in destroying phage. Twort, Baker, Finn & Powell(14) have shown that 10 % resorcinol dissolved in propylene glycol produced satisfactory bacterial reduction, whilst Robertson, Bigg, Puck & Miller(15) and Challinor & Duguid(16) have shown that propylene glycol, too, can be effective.

The figures given in Table 15 show that when a solution of 10 % resorcinol was sprayed for 10 min. to give a concentration of 0·014 p.p.m. into air containing phage 144*f* (test 28), it destroyed nearly all the particles 16 min. after phage spraying ceased. It will be seen that the 10 % survival of phage 10 min. after phage spraying ceased is only temporary. With phage *y*1 (test 47), the phage particles survived when a mist containing resorcinol was sprayed into the air.

In tests 43 and 48 propylene glycol failed to destroy phage 144*f*. Similarly, test 44 shows that phage *y*1 survived after spraying propylene glycol into the air.

The good recoveries of phage *y*1, after spraying resorcinol into the atmosphere containing phage, and of phages 144*f* and *y*1 after spraying propylene glycol, show that neither of these substances was toxic to the two phages at the concentrations used. It is unlikely that the great reduction in the numbers of particles of phage 144*f* shown after introducing resorcinol into the air, was due to an inhibitory or destructive effect caused by resorcinol on the phage deposited on the gelatine or agar plates after exposure to air had ceased.

Table 14. *The germicidal effect on phage of spraying hypochlorite solutions into the air immediately after the spraying of phages* 144*f*, *y*1 *and* 236/9 *had ceased, at different levels of relative humidity*

Phage used ...		144 *f*												
Test no. ...		50	49		52	51		54	53		56	55		
Concentration of hypochlorite solution (% av./cl.)		Control	10		Control	10		Control	11·4		Control	11·4		
Approx. concentration of hypochlorite mist (p.p.m. av./cl.)		—	0·01		—	0·01		—	0·013		—	0·011		
Concentration of phage preparation per ml.		10^9	10^9		10^9	10^9		10^9	10^9		10^9	10^9		
Approx. concentration of phage particles sprayed per 10^3 l.		17×10^7	17×10^7		18×10^7	17×10^7		17×10^7	17×10^7		14×10^7	14×10^7		
Method of recovery	Time after phage spraying ceased (min.)	Average no. of plaques per plate		% survival			% survival			% survival			% survival	
(1) Sedimentation on agar plates	10	∞ ∞ ∞	783	—	∞ ∞	2	—	∞ ∞ ∞	699	—	∞ ∞	24	—	
	20	∞ ∞ ∞	579	—	∞	0	0	∞ ∞ ∞	600	—	∞	2	—	
	40	∞	269	—	∞	0	0	∞	341	—	1280	1	0·1	
	55	∞	153	—	603	0	0	∞	118	—	517	0	0	
	65	1628	90	6	473	0	0	1703	54	3	222	.	—	
	75	1064	.	—	179	.	—	1349	51	4	68	.	—	
		No. of plaques per plate												
(2) Aspiration through slit sampler on to agar plates	14	∞ ∞ ∞	∞ ∞	—	∞ ∞ ∞	2	—	∞ ∞ ∞	∞ ∞ ∞	—	∞ ∞ ∞	2	—	
	30	∞ ∞ ∞	∞ ∞	—	∞ ∞ ∞	0	0	∞ ∞ ∞	∞ ∞ ∞	—	∞ ∞ ∞	0	0	
	50	∞ ∞ ∞	∞	—	∞ ∞ ∞	0	0	∞ ∞ ∞	∞	—	∞ ∞ ∞	0	0	
	70	∞ ∞ ∞	2100	—	∞ ∞	.	—	∞ ∞ ∞	1832	—	∞ ∞ ∞	.	—	
		Concentration of phage per ml. of gelatine												
(3) Aspiration through slit sampler on to gelatine plates	0	10^5	—	—	10^4	—	—	10^5	—	—	—	—	—	
	10	10^4	10^3	10·0	10^4	10^1	0·1	10^5	10^3	1·0	10^4	****	0	
	16	10^4	10^3	10·0	10^4	***	<0·1	10^5	10^4	10·0	10^4	****	0	
	24	10^4	10^3	10·0	10^4	****	—	10^6	10^3	0·1	10^4	****	0	
	35	10^4	10^3	10·0	10^3	****	—	10^5	10^2	0·1	10^3	****	0	
	45	10^4	10^3	10·0	10^4	****	—	10^5	10^2	0·1	10^2	****	0	
	60	10^4	10^2	1·0	10^4	****	—	10^4	10^2	1·0	**	.	—	
	75	10^4	10^3	10·0	10^3	.	—	10^4	10^2	1·0	**	.	—	
Temp. of room, °F.		80	81		81	81		79	78		81	81		
Relative humidity		50–56	47–56		62–72	60–71		47	50–54		80–81	85–96		

Table 14 (*continued*)

Table 14 (*continued*)

Phage used ...		*y*1					236/9				
Test no. ...		58	57	59	61	60	63	62	65	64	
Concentration of hypochlorite solution (% av./cl.)		Control	10	Distilled water	Control	10	Control	10	Control	10	
Approx. concentration of hypochlorite mist (p.p.m. av./cl.)		—	0·011	—	—	0·01	—	0·014	—	0·014	
Concentration of phage preparation per ml.		10^8	10^8	10^8	10^8	10^8	10^8	10^8	10^8	10^8	
Approx. concentration of phage particles sprayed per 10^3 l.		21×10^6	18×10^6	21×10^6	14×10^6	14×10^6	2×10^7	2×10^7	2×10^7	2×10^7	
Method of recovery	Time after phage spraying ceased (min.)	Average no. of plaques per plate									
(1) Sedimentation on agar plates	10	∞ ∞	∞	—	∞ ∞	∞	39	—	∞ ∞	∞ ∞	—	∞ ∞	0	0
	20	∞	∞	—	∞	∞	8	—	∞ ∞	∞ ∞	—	∞	0	0
	40	* ∞	*923	—	1274	*1019	*0	0	∞ ∞	∞	—	67	0	0
	55	*1280	*704	55	1130	*720	.	—	∞	653	—	8	.	—
	65	.	.	—	.	.	.	—	.	.	—	.	.	—
	75	.	.	—	.	.	.	—	.	.	—	.	.	—
		No. of plaques per plate									
(2) Aspiration through slit sampler on to agar plates	14	∞ ∞	∞ ∞	—	∞ ∞	∞ ∞	16	—	∞ ∞	∞ ∞	—	∞ ∞	0	0
	30	∞ ∞	∞	—	∞ ∞	∞ ∞	30	—	∞ ∞	∞ ∞	—	∞ ∞	0	0
	50	.	.	—	.	.	.	—	∞ ∞	∞ ∞	—	∞	.	—
	70	.	.	—	.	.	.	—	.	.	—	.	.	—
		Concentration of phage per ml. of gelatine									
(3) Aspiration through slit sampler on to gelatine plates	0	10^6	—	—	—	10^4	—	—	10^5	—	—	10^6	—	—
	10	10^5	10^5	100	10^5	10^5	****	—	10^5	10^5	100	10^3	10^3	100
	16	10^4	10^4	100	10^5	10^4	****	—	10^4	10^5	100	10^4	****	0
	24	10^6	10^4	1·0	10^4	10^4	****	—	10^3	10^4	10·0	10^3	****	0
	35	10^4	10^4	100	10^4	10^4	****	—	10^5	10^5	100	10^3	Found in 1 ml.	<0·1
	45	10^4	10^4	100	10^4	10^4	****	—	10^4	10^4	100	10^2	****	0
	60	.	.	—	.	.	.	—	10^5	10^3	1·0	10^3	.	—
	75	.	.	—	.	.	.	—	.	.	—	.	.	—
Temp. of room, °F.		77	77		79	82	81		82	81		86	86	
Relative humidity		44–52	42–52		44–50	80–86	70–78		42–63	43–61		76–89	74–86	

Key: · = no test. * = these plates were exposed respectively at 37 and 50 min. after phage spraying ceased. ** = not found in 10^2. *** = not found in 10^1. **** = not found in 1 ml.

Table 15. *The germicidal effect on phage of spraying a solution of resorcinol and of propylene glycol into the air immediately after the spraying of phages 144f and y1 had ceased*

Disinfectant used ...		10 % resorcinol				Neat propylene glycol					
Phage used ...		144 *f*		*y*1		144 *f*				*y*1	
Test no. ...		16 Control	28	41 Control	47	38 Control	43	46 Control	48	41 Control	44
Approx. concentration of disinfectant mist (p.p.m.)		—	0·014	—	0·014	—	0·13	—	0·07	—	0·1
Concentration of phage preparation per ml.		10^{10}	10^{10}	10^{8}	10^{8}	10^{9}	10^{9}	10^{10}	10^{10}	10^{8}	10^{8}
Approx. concentration of phage particles sprayed per 10^{3} l.		16×10^{6}	14×10^{6}	14×10^{6}	14×10^{6}	14×10^{6}	12×10^{6}	12×10^{6}	12×10^{6}	14×10^{6}	16×10^{6}
Method of recovery	Time after phage spraying ceased (min.)	Average no. of plaques per plate									
(1) Sedimentation on agar plates	10	∞ ∞	1073	—	∞	∞	—	∞	∞	—	∞	∞	—	∞	∞	—
	20	∞ ∞	35	—	26	685	—	671	1356	—	∞	∞ ∞	—	26	514	100*
	40	∞ ∞	3	—	4	24	—	25	87	—	983	1110	100*	4	34	100*
	55	1433	0	0	1	4	—	6	24	—	686	277	40	1	4	100*
	60	789	0	0	.	.	—	0	4	—	405	273	67	.	.	—
	75	476	3	0·6	.	.	—	.	.	—	.	.	—	.	.	—
		No. of plaques per plate														
(2) Aspiration through slit sampler on to agar plates	14	∞ ∞	563	—	∞	∞	—	∞	.	—	∞ ∞	∞ ∞	—	∞	.	—
	30	∞ ∞	0	0	17	61	—	∞	.	—	∞ ∞	∞ ∞	—	17	.	—
	50	∞	0	0	.	.	—	130	.	—	∞	∞	—	.	.	—
	70	∞	0	0	.	.	—	.	.	—	.	.	—	.	.	—
		Concentration of phage per ml. gelatine														
(3) Aspiration through slit sampler on to gelatine plates	0	10^{4}	—	—	10^{4}	—	—	10^{4}	—	—	10^{5}	—	—	10^{4}	—	—
	10	10^{4}	10^{3}	10·0	10^{3}	10^{4}	100*	10^{4}	.	—	10^{4}	10^{5}	100*	10^{3}	.	—
	16	10^{5}	10^{1}	0·01	10^{3}	10^{3}	100	10^{3}	.	—	10^{4}	10^{4}	100	10^{3}	.	—
	24	10^{4}	10^{1}	0·1	10^{2}	10^{2}	100*	10^{3}	.	—	10^{4}	10^{5}	100*	10^{2}	.	—
	35	10^{4}	***	<0·1	Found in 1 ml.	10^{2}	100*	10^{3}	.	—	10^{4}	10^{5}	100*	Found in 1 ml.	.	—
	45	10^{5}	***	<0·01	10^{1}	Found in 1 ml.	10	10^{3}	.	—	10^{4}	10^{3}	10	10^{1}	.	—
	60	10^{4}	***	<0·1	.	.	—	10^{3}	.	—	10^{3}	10^{3}	100	.	.	—
	75	10^{3}	***	<1·0	.	.	—	.	.	—	.	.	—	.	.	—
Temp. of room, ° F.		34	47	48	41	42	48	40	42	48	38
Relative humidity		84	88	88	86	86	94	86	86	88	88

Key: · = no test. *** = not found in 10^{1}. 100* = approximately 100 %.

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The experiments with resorcinol and propylene glycol were few and do not warrant final conclusions on their usefulness as germicidal mists to destroy air-borne phage. On grounds of cost and availability hypochlorite solutions appear to be more economical and useful for spraying as germicidal mists in order to destroy phage in the air than either of these other two substances at present. Hypochlorite is also basically innocuous.

VII. *Extent of pungent odour of mists*

In most of the experiments, spraying of hypochlorite solutions of different strengths lasted for 10 min. only and it is difficult to predict what the extent of the odour and the irritating effect of the mist on the throat and eyes would have been had the hypochlorite solutions been sprayed for longer intervals. Solutions giving a concentration of 0·00014 and 0·0014 p.p.m. av./cl. when sprayed into the air produced a mist with practically no smell or irritant effect, and stronger hypochlorite solutions giving a concentration of up to 0·02 p.p.m. av./cl. gave rise to mists with a definite and pungent smell, but these mists did not inconvenience an operator working in the room directly after the spraying of hypochlorite solution had ceased.

Hence when spraying the strongest brands of commercial hypochlorite solutions available (i.e. containing 10–15 % av./cl.), the usefulness of these mists for destroying air-borne phage during the limited periods of immunity from contamination required for starter preparation, would not be reduced by objectionable or irritating smell. When respraying with hypochlorite solution every ½ hr. is necessary to combat the danger of continuous recontamination of the atmosphere with air-borne phage, the operator might find the accumulative odour pungent. In this connexion, Elford & van den Ende (11) have reported that people will breathe atmospheres containing hypochlorous acid gas in 0·1 p.p.m. without comment, and it is probable that with intermittent spraying at ½ hr. intervals, the mist would not be irritating.

Discussion

An extensive literature now exists on the germicidal action of hypochlorites, glycols and various phenolic compounds when sprayed as mists on air-borne bacterial organisms. The efficiency of hypochlorite mists has been demonstrated by Douglas, Hill & Smith (17), Masterman (18), Baker, Finn & Twort (19), Challinor (10), and Pulvertaft (20), whilst Twort and his co-workers (14) have also shown that resorcinol and its related compounds may also be effective when sprayed as mists against bacteria. Robertson *et al.* (15) found that propylene glycol itself is an efficient germicide. Recent work with these compounds has proved that they may be equally effective against influenza virus. Thus Edwards & Lidwell (21), in this country, have shown that hypochlorite mists were very effective in the destruction of influenza virus, while in America, Henle & Zellat (22) and Stokes & Henle (23) were able to confirm a similar action for propylene glycol on the same virus.

No previous reference to quantitative experimental data could be found for any of these aerial disinfectants against air-borne bacteriophage. As mentioned earlier, Hunter & Whitehead (1) have shown that hypochlorites inactivated bacteriophage in experiments with *Str. cremoris in vitro* and suggested that air-borne phage might be combated by spraying with active chlorine. The data presented demonstrate that mists prepared from

hypochlorite solutions are as effective against atomized bacteriophage as other workers have shown them to be germicidal against influenza virus or bacteria in the air.

The experimental data show that a hypochlorite mist with a low concentration of 0·0009–0·00014 p.p.m. av./cl. reduces the survival of particles of bacteriophage to 1–10 %. In practice such a mist does not retain its germicidal properties for long and is therefore not of sufficiently high concentration of available chlorine to combat the reinfection of the atmosphere by air-borne bacteriophage, which would occur continuously at cheese-making factories. The poor persistency of the germicidal action of hypochlorite mists produced by spraying solutions containing 1 % av./cl. has also been noted by Baker *et al.* (19) and by Elford & van den Ende (11). The spraying of solutions stronger than 5 % av./cl., to give the mist a concentration of at least 0·003 p.p.m. av./cl. is essential. If the air of the laboratory or bulk-starter room at a cheese factory is treated with a mist of this concentration, there should usually be a sufficient margin of germicide to inactivate air-borne phage during critical operations in the propagation of starter. If these operations occupy more than ½ hr., it will be advisable to respray with hypochlorite solution. A fine mist, therefore, produced by spraying 4 ml. of full strength 'approved' solution (9–12 % av./cl.) for every 1000 cu.ft. of air space will give protection from phage contamination.

Baker & Twort (24) and more recently Elford & van den Ende (11) have drawn attention to the importance of humidity in controlling the efficiency of hypochlorite aerosols in bacterial destruction. The experiments at higher temperatures given in this paper have demonstrated the importance of a high relative humidity in phage destruction too. It seems that below a relative humidity of 50 % phage destruction is slight or negligible irrespective of the strength of hypochlorite solution used.

The results obtained at low (35–45° F.) and high (77–86° F.) temperatures show that in the former phage destruction was satisfactory and the prevailing relative humidity was about 80 %. At the higher temperatures phage destruction was not satisfactory until a relative humidity of about 50 % had been reached. A consideration of the actual quantities of water vapour present at these various temperatures (Smithsonian tables) shows that at 41° F. and at a relative humidity of 80 %, 5·4 g. of water vapour are present per cu.m. On the other hand, at 80° F. and a relative humidity of 50 %, 12·7 g. of water vapour are present per cu.m. Thus, although at 80° F. and a relative humidity of 50 % little or no destruction of phage takes place, at 41° F. with only 5·4 g. of water vapour per cu.m. and a relative humidity of 80 % the germicidal efficiency is satisfactory. Thus it would seem that it is the relative and not the absolute humidity which is important, although it appears that Elford & van den Ende (11) hold the opposite view. In dairy practice where free water and steam is invariably present, a relative humidity of 70 % or higher may readily be assumed to be present, and the effectiveness of hypochlorite mists would thus be enhanced.

Summary

1. The germicidal effect of spraying hypochlorite solutions of varying strength on three different phage races of streptococci from cheese starter has been described. It has been shown that fine mists containing 0·003–0·02 p.p.m. av./cl., usually ensure satisfactory destruction of air-borne phage.

2. Below a relative humidity of about 50 % hypochlorite solutions cause a slight or negligible destruction of bacteriophage particles.

3. A gelatine medium has been found useful for collecting phage particles from the air, since it could be liquefied without danger of destruction of the phage after exposure and a quantitative estimation obtained of the phage particles collected.

4. When the relative humidity was low the recovery of phage particles from the air was increased.

5. Some experiments using mists of resorcinol and propylene glycol are also reported.

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336. SURFACE ACTIVITY AND PROTEINS OF MILK

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(With 7 Figures)

Though 40 years have passed since attention was first drawn to the surface activity of milk (1, 2), comparatively few investigations (3-14) have been published regarding the conditions at the milk/air interface, conditions which are of importance in phenomena such as the foaming of milk.

Normal cows' milk is a moderately surface-active system, its surface tension (σ) amounting to approximately two-thirds of that of water. Its surface activity has been attributed, without much evidence, to the presence of one or more of the milk proteins, but it is not known which of them are concerned in building up the surface layer. Consequently, no conception can be formed of the structural arrangements within the layer. These arrangements are doubtless influenced by the presence of the fat globules, but the manner in which the fat exerts its influence is far from well understood.

Several observers (4, 7, 10) have stated that milk may be considerably diluted with water without any marked change in its σ-value. This finding suggests—as will be confirmed below—that milk has a surface tension-concentration relationship of the kind typical for many surface-active systems: in high dilutions σ falls rapidly with increasing concentration, whilst little further change occurs in more concentrated solutions. Procedures causing a partial removal or alteration of the surface-active material present in undiluted milk can, therefore, not be expected to affect the σ-value markedly, and it is not surprising that such work as has been done on the effect on σ of factors known to cause alterations in the physical or chemical make-up of milk should, in general, have failed to give striking results. The effect of such alterations should, however, become apparent when surface tension-concentration or surface tension-dilution curves are considered in place of the σ-values of the undiluted fluid, and this has been done in the present investigation.

Experimental

Two methods have been most commonly used to measure the surface tension of milk, Traube's stalagmometric method, and du Nouy's ring method. Mohr & Brockmann (11) have shown that the results obtained by the semi-dynamic stalagmometer method are somewhat higher than those given by the static ring method, but show, in general, parallelism. Belle (5) has demonstrated that with du Nouy's method considerable time is needed to reach a steady σ-value, and since, according to Mohr & Brockmann, the surface of milk is altered by membrane formation (*Häutchenbildung*) on ageing, it is doubtful whether, with milk, a 'static' measure of σ is any more 'true' than one obtained on a fresh surface. In fact, it can be argued that a measure of the latter may be more valuable, since in foam formation, for instance, interest centres on a fresh rather than on an aged

surface. These considerations, coupled with the demand for simplicity and rapidity in serial measurements, have led to the adoption of the stalagmometer method, although its limitations are fully realized.

Tests were carried out at room temperature in the usual manner(11). A value of $\sigma = 73$ dynes/cm. for the surface tension of water was used in all calculations. Time and labour were saved by the observation that it was practicable to omit cleaning of the stalagmometer with chromic acid and water between runs when dealing with various dilutions of the same fluid, provided that measurements were started with the most dilute solution of the series, and continued with solutions of increasing concentration.

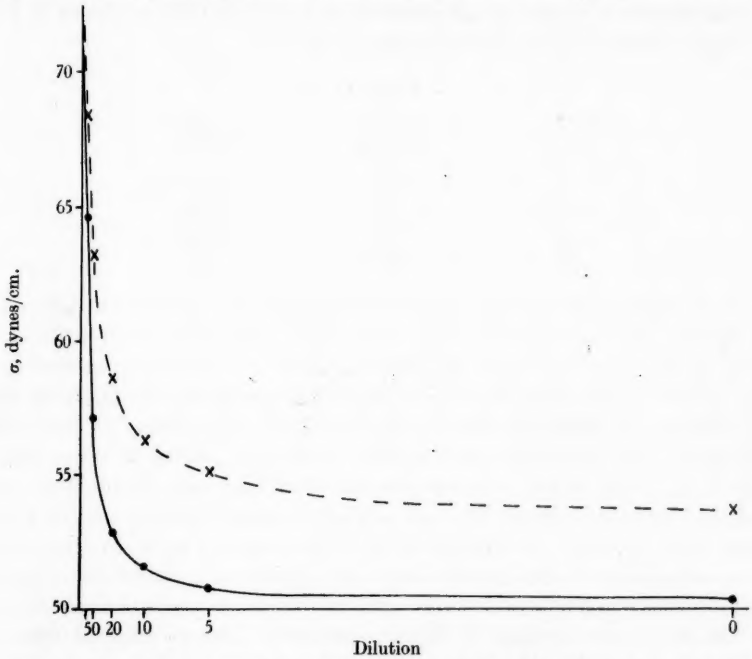

Fig 1. Effect of dilution on σ (mean of 16 experiments): ●——● whole milk; × – – × separated milk.

All milk samples consisted of mixed milk of the Institute cows. Separated milk was prepared in a commercial separator to a fat content of less than 0·05%. All samples containing more fat were cooled to 0–5° C. for at least 2 hr. prior to testing, so as to allow for the full lowering of the surface tension known to be caused by the solidification of the fat(3, 6, 11). These samples were warmed to room temperature before testing.

RESULTS

I. *The effect of dilution on the surface tension of whole and separated milk. The influence of fat*

Surface tension-dilution curves were obtained for sixteen samples of whole and separated milk. The σ of the undiluted fluids varied between 46·6 and 52·7 dynes/cm. for whole milk, and between 51·9 and 56·6 dynes/cm. for separated milk. The results of the dilution experiments are summarized in Fig. 1, giving the mean values for each dilution.

As suggested above, the relation between surface tension and dilution is of the type most commonly encountered with capillary-active solutions: a high degree of dilution is needed, particularly with whole milk, to cause drastic changes in σ. It appears as if milk contained surface-active substances in far higher concentrations than are needed to form what might be termed a 'saturated' surface layer. On that basis, however, it is difficult to understand why samples of undiluted separated milk should show appreciable differences in σ, unless it is the type rather than the quantity of surface-active material which varies from sample to sample.

It was found that, with whole milk of known surface tension (σ_0), the surface tension (σ_n) at any given dilution (n) could be calculated from $\sigma_n = \sigma_0 + 0{\cdot}135n$, as shown in Table 1. No such simple relationship was found for separated milk.

Table 1

Dilution n	σ found dynes/cm.	σ_n (calc.) dynes/cm.
0	50·3	—
5	50·8	51·0
10	51·6	51·65
20	52·9	53·0
50	57·1	57·05
100	64·6	63·8

Fig. 1 shows that on the average undiluted separated milk lowers the surface tension of water by 3·4 dynes/cm. less than whole milk. Such a relatively small difference could perhaps be explained on the basis of differences in the concentration of surface-active material. However, the σ-dilution curves show that conditions are far more complex than indicated by the difference between the undiluted fluids. The fact that undiluted separated milk shows less surface activity than whole milk diluted 20 times with water can hardly be explained as due to a mere concentration difference, and other factors must be considered. In whole milk the fat itself may play a specific role by causing a different orientation of the molecules or micelles forming the boundary layer. Alternatively, the substances surrounding the fat globules may exert an influence. Moyer (15), in his electrophoretic study, has found indications that 'whatever the outer coating (of the fat globules) may be, the membrane complex of Palmer apparently becomes exposed upon simple dilution, provided that the pH is held between 6·0 and 7·5'.

The complications due to the presence of fat are illustrated by the following experiments dealing with the influence of variations in the fat content on the surface tension of milk. In cream, σ is known to decrease slightly with increasing fat concentration (11), but few data are available for milk containing less fat than whole milk. Experiments were therefore carried out with whole milk, separated milk and mixtures of the two. Typical results are presented in Fig. 2, which show that the fall in σ is not proportional to the concentration of fat, but most marked for small percentages of fat.

When the effect of dilution on the surface tension of these mixtures was studied, it was found that this lack of proportionality persisted, as demonstrated by the example in Fig. 3.

As the limit of the effect of the fat addition did not seem to be reached in the higher dilutions, the range was extended in experiments with suitable mixtures of cream and separated milk. Fig. 4 shows the result of one such experiment, σ at various dilutions being plotted against the fat percentage of the undiluted mixtures.

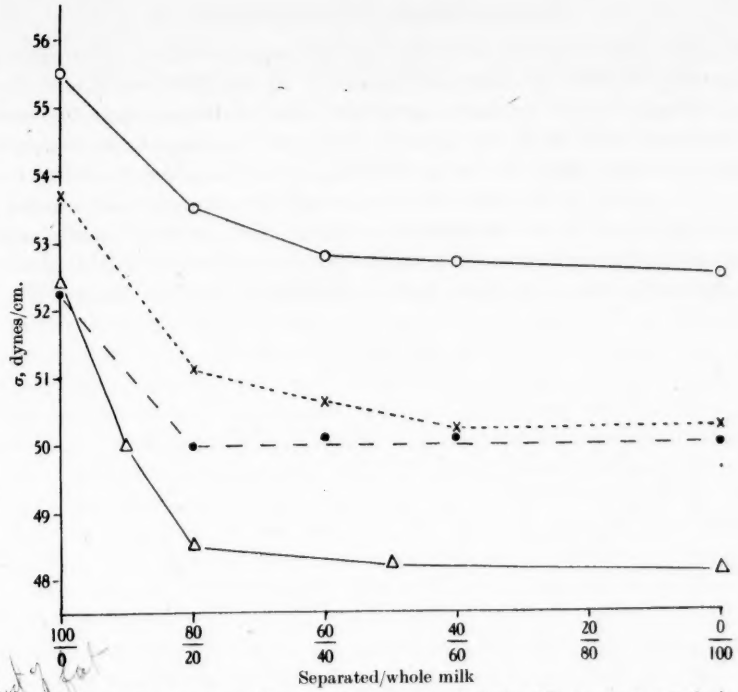

Fig. 2. Surface tension. Mixtures of separated and whole milk: ○——○ sample A. ×----× sample B. ●——● sample C. △——△ sample D.

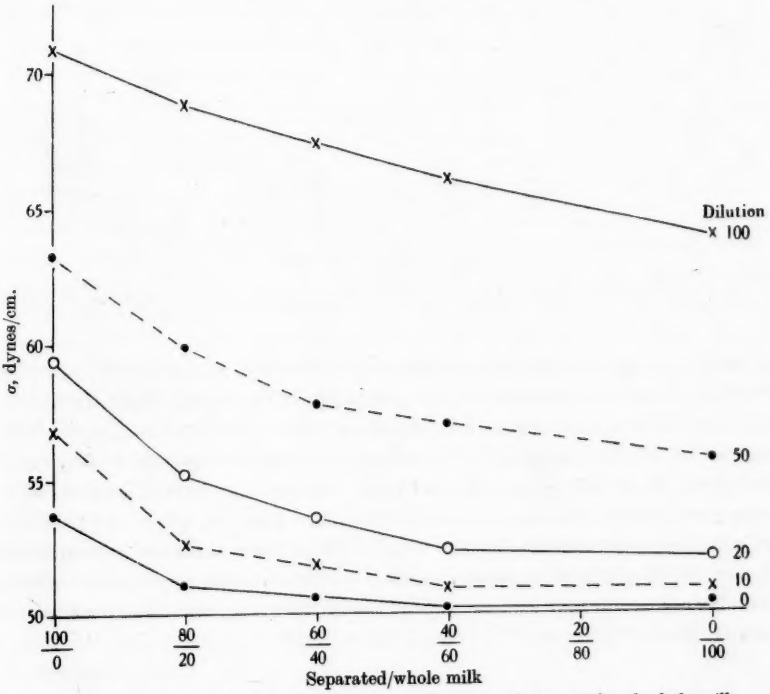

Fig 3. Effect of dilution on surface tension. Mixtures of separated and whole milk.

It is seen that even when milk is diluted 100-fold, small additions of fat cause a more marked lowering of σ than do subsequent additions. If the effect were due to the introduction of a surface-active compound associated with the fat, such as the membrane protein, one would, with highly diluted milk, expect the lowering of σ to be proportional to the amount of fat added. It seems, therefore, more likely that the effect is due to changes in the spacing of the surface-active material of separated milk, induced by the introduction of the fat. In that case one can visualize that, as the fat content increases, the surface-active material assumes the most effective orientation, so that the addition of fat beyond a certain concentration cannot result in any further changes. The arrows

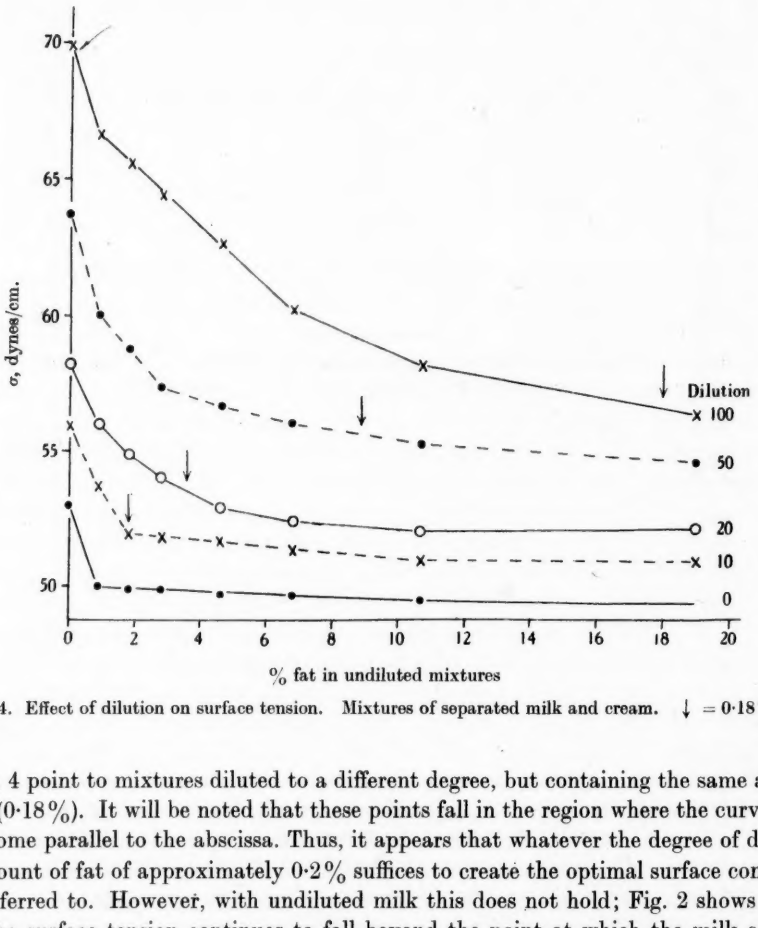

Fig. 4. Effect of dilution on surface tension. Mixtures of separated milk and cream. ↓ = 0·18% fat.

in Fig. 4 point to mixtures diluted to a different degree, but containing the same amount of fat (0·18%). It will be noted that these points fall in the region where the curves tend to become parallel to the abscissa. Thus, it appears that whatever the degree of dilution, an amount of fat of approximately 0·2% suffices to create the optimal surface conditions just referred to. However, with undiluted milk this does not hold; Fig. 2 shows clearly that the surface tension continues to fall beyond the point at which the milk contains 0·2% of fat. (Assuming a fat content of 4% for whole milk, this point would be reached in a mixture of 95 parts of separated with 5 parts of whole milk.) The difference in behaviour between diluted and undiluted milk remains for the present without explanation.

II. *The nature of the surface-active material in separated milk*

The foregoing experiments have shown that the conditions at the boundary milk/air are complicated by the presence of fat. As the changes caused by the fat are likely to be of a physical rather than of a chemical nature, subsequent work was done with separated milk which represents a simpler system.

As mentioned in the introduction, little is known about the nature of the surface-active material in milk. Behrendt (4) has shown that milk sera obtained by precipitation with colloidal iron are far less surface active than the original milk, but apart from this observation the statement that the surface activity of milk is due to the presence of the proteins is based on assumption rather than on experimental evidence. A search of the literature fails to produce any proof even that the surface activity of milk is due exclusively to constituents of a colloidal character. Such proof can be easily obtained by dialysis experiments, as shown by the following example: Two collodion tubes containing 25 ml. of distilled water were inserted into 150 ml. samples of separated milk, and kept for 48 hr. at 5° C. After this period the skim milk was found to contain 76 and 75 mg. % of chloride, whilst the clear, faintly greenish solutions inside the collodion bags contained 80 and 79 mg. % of chloride. Equilibrium had thus been established. The separated milk had a surface tension of 52·2 dynes/cm. against 52·1 in the original milk, whilst the contents of the collodion bags gave values of 72·1 and 68·4 dynes/cm. As the separated milk gave a surface tension of 67·2 dynes/cm. when 100 times diluted, it may be concluded that the surface-active material in milk is almost entirely of a colloidal character.

Since the proteins form the bulk of the colloidal components of separated milk, one or more of them are likely to be responsible for the surface activity. As mentioned before, Behrendt (4) observed that the serum obtained by adding colloidal iron to milk is far less surface active than the original milk. It can also be shown that sera obtained by reagents causing a very complete precipitation of the proteinaceous components of milk are hardly surface active. Thus, a serum prepared by adding 10 ml. of a 1·57% uranyl acetate solution to 10 ml. of milk, made up to 100 ml. with water, had a surface tension of 69·3 dynes/cm. as compared with 71·8 dynes/cm. for a 'blank' (no milk added). Sera prepared by precipitation with alkaline zinc sulphate gave similar results. Though not absolutely conclusive, since colloids other than proteins may be co-precipitated, these findings suggest strongly that the surface activity of milk is due to the presence of certain proteins or protein-like substances. Of these, separated milk contains casein, lactalbumin, lactoglobulin, and a rather ill-defined proteose-peptone fraction, and the question arises as to their specific role in the surface-active behaviour of milk. Data for the surface activity of proteins isolated from separated milk have recently been provided by El-Rafey & Richardson (16), who measured the surface tension on freshly stirred surfaces by du Nouy's method at 25° C. According to their results, sols of Ca-caseinate and lactalbumin of a protein content equivalent to their concentration in milk (2·6 and 0·3% respectively) have a surface tension of the same order as separated milk (*c.* 53 and 55 dynes/cm. respectively), whilst a sol of lactoglobulin (0·13%) proved to be less surface active (*c.* 57·5 dynes/cm.). From this it would appear that casein and lactalbumin are likely to be the fractions mainly responsible for the surface activity of milk. However, dilution affects these sols in a manner so different from milk that caution is needed in drawing any conclusions. Whereas separated milk in a dilution 1 : 100 shows little residual activity (see

Fig. 1), the sols of Ca-caseinate and lactalbumin when similarly diluted (to 0·026 and 0·003% of protein respectively) were found to be still markedly surface active (*c.* 55 and 59 dynes/cm. respectively). Thus, each of these sols was far more surface active than separated milk containing both these proteins in similar concentration. This suggests that, in spite of the care taken in their preparation, the sols differed in their properties from the behaviour of the proteins in untreated milk. In the present study, the problem was approached from a different angle, namely, by investigating the behaviour of the residual fractions obtained by the removal of one or more of the protein fractions from separated milk.

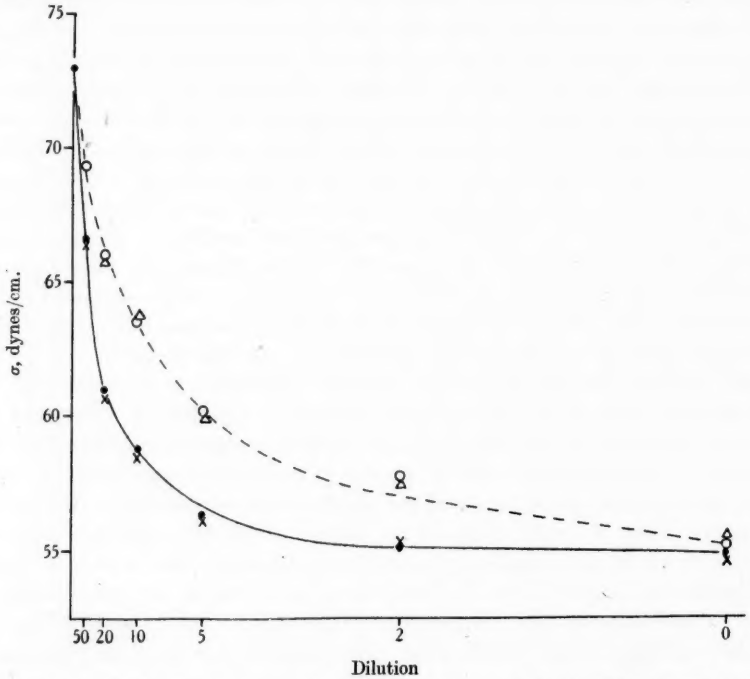

Fig. 5. ● Separated milk. ○ Rennet whey. × Rennet whey at *p*H 4·4–4·6. △ Rennet whey from separated milk heated to 95° C.

III. *Effect of the removal of casein on σ*

(*a*) *Removal by rennet coagulation*

Mohr & Brockmann (11) have observed that whey obtained by rennet coagulation of separated milk had the same surface tension as the original milk. They concluded that the casein complex is without influence on the surface tension, a conclusion which appears *a priori* unlikely, since more than four-fifths of the milk proteins consists of casein. It was confirmed by a number of experiments that the σ of rennet whey differs little from that of the original milk. It was found, however, that on dilution such whey loses its surface activity more rapidly than milk, as illustrated in Fig. 5 (● and ○). This indicates that casein does, as one would expect, contribute to a far from negligible extent to the surface activity of milk.

(*b*) *Removal by acid coagulation*

When the casein was removed by acid precipitation (with acetic acid and sodium acetate, as described by Rowland (17), but reducing the final volume to twice that of the milk), a rather surprising result was obtained: unlike rennet whey the casein-free filtrate gave a σ-dilution curve which was practically identical with the curve obtained for the original milk. The increased surface activity of the casein-free filtrate was found to be due to its lower *p*H: on adjustment of the *p*H, by addition of alkali, to that of the milk, the same type of σ-dilution curve resulted as shown for rennet whey in Fig. 5. Similarly, the σ of rennet whey was found to fall on addition of acid and reached a minimum at a *p*H of about 4·5, further addition of acid causing a slow rise of σ. The σ-dilution curve of rennet whey adjusted to approximately *p*H 4·5 was, again, practically identical with that of the untreated milk ($\times$ in Fig. 5).

IV. *Effect of the removal of the heat-coagulable proteins on* σ

Boiling of milk leads to denaturation of the lactalbumin and lactoglobulin. This has been found to be without effect on the surface tension (1, 11), and experiments showed that the σ-dilution curve of boiled milk does not differ materially from that of unheated milk. To study the effect of the removal of the heat-coagulable proteins, two procedures were adopted:

(1) Casein-free filtrates were adjusted to *p*H 4·75–4·8 and the proteins coagulated by boiling (according to Rowland (17) coagulation in this manner is incomplete).

(2) Separated milk was heated to 90–95° C. for 15 min., and the denatured proteins co-precipitated with casein by either acid coagulation or rennet coagulation (with addition of $CaCl_2$ solution (60 mg. Ca/100 ml. milk) to aid formation of a firm curd).

Whatever procedure was chosen, the resulting sera gave σ-dilution curves which were practically identical with those obtained in the presence of lactalbumin and lactoglobulin (an example is shown in Fig. 5 ($\triangle$)). These results justify the conclusion that the heat-coagulable proteins have little or no influence on the surface conditions of milk.

V. *The residual protein fraction*

It follows that separated milk still possesses considerable surface activity after the removal of the casein, lactalbumin and lactoglobulin, or in other words, that milk contains a surface-active component other than its principal proteins. The work of Ansbacher *et al.* (18) suggested the possible existence of such a fraction: they found that a surface-active substance can be obtained 'by precipitation with chlorine gas from various milk sera from which the casein alone, or the casein and lactalbumin, or casein, lactalbumin and lactoglobulin had been removed'.* The question whether milk contains proteins or protein-like substances other than casein, lactalbumin and lactoglobulin has been considered by several authors (see Rowland (19)). The uniformity of the surface-active behaviour of milk sera obtained by different procedures (acid and rennet coagulation) makes it seem unlikely that the residual fraction constitutes an artefact, a possibility considered by Osborne & Wakeman (20). Whether the fraction consists of a true protein or of a proteose or peptone compound cannot be decided on the basis of the evidence so far presented. Its nature was, therefore, investigated in some detail.

* The chlorination product was not investigated in detail.

In order to obtain data on the quantities of residual fraction present in milk, sera freed from casein and the heat-coagulable proteins were subjected to dialysis. To compare the σ-dilution curves before and after dialysis, use was made of the fact that σ reaches a minimum at a *p*H of approximately 4·5. The comparison of sera adjusted to this *p*H showed that the surface activity remained unimpaired by the dialysis. Table 2 contains data for the distribution of nitrogen in two milk samples.

Table 2. *Nitrogen distribution*

N (Kjeldahl) mg./100 ml. milk	Rennet-coagulated milk	Acid-coagulated milk
Total	534	580
After removal of casein and heat-coagulable proteins	50·0	53·6
Dialysable	33·3	36·9
Residual fraction	16·7	16·7

These data show that the marked surface activity of milk freed from its main proteins is due to a fraction, or parts of a fraction constituting no more than about 3% of the total protein. From multiplication by the customary factor (6·38) it follows that milk probably contains about 0·1% of this fraction.

The dialysed sera give a faint biuret test, and become slightly turbid on acidification to a *p*H of about 4·5, the turbidity resolving on addition of further acid. The sera show a marked foaming tendency. They can be concentrated by evaporation to between one-half and one-third of their volume without loss in surface activity, but on further concentration such a loss becomes noticeable.

VI. *Isolation of the surface-active material* (σ-*proteose*)

Methods for concentrating the surface-active material in the residual fraction were based on the observation that it can be quantitatively removed by half-saturation with ammonium sulphate. Tests showed that the filtrate from the material precipitated by half-saturation gave no further precipitate on addition of ammonium sulphate to saturation, and was surface inactive after removal of the salts by dialysis.

The procedure adopted for the concentration of the surface-active material was to stir ammonium sulphate into the serum obtained after the removal of the casein and heat-coagulable proteins until half-saturation was reached. After standing for at least 1 hr., the solutions were centrifuged, the supernatant decanted, and the precipitate dispersed in water, using about one-tenth of the volume of the original serum. The aqueous dispersion was centrifuged to remove impurities, the supernatant half-saturated with ammonium sulphate and the precipitate recovered as described above. After repeating the process, a pure white precipitate was obtained. This was triturated with water to a thin paste which was transferred to a seamless cellophane bag. The contents, layered with toluene, were dialysed first for 12–18 hr. against running tap water, then for 2–3 days at room temperature against slow-dripping distilled water. The white paste takes up water during the initial stages of the dialysis, and changes to a faintly turbid solution; after prolonged dialysis the turbidity increases slightly, but no precipitate is formed.

In this manner, concentrates containing between 1·0 and 1·5% of the protein material were obtained. From these the dry material was isolated in two ways:

(1) *By precipitation with alcohol-ether mixture.* The concentrate was stirred into eight times its volume of a slightly acid mixture of 3 parts of alcohol with 1 part of ether (1 drop

of *N* acetic acid per 100 ml. of mixture). The mixture was brought to the boil, left to cool, and the flocculant precipitate filtered off. After washing with absolute alcohol, it was dried *in vacuo* over $CaCl_2$. After 2 days' drying, the brittle white material was pulverized, and the drying continued to constant weight. The dry powder is hygroscopic taking up about 10% of moisture when exposed to the air. On evaporation of the alcohol-ether filtrate, a very small amount of fatty material remained. Tests for phospholipoids in this residue gave a negative result.

(2) *By freeze-drying.* Dr E. C. Bate-Smith (Cambridge), to whom the author wishes to express his indebtedness, kindly arranged for the freeze-drying of a 120 ml. sample of dialysed concentrate. By this process the material is obtained as a very light, bulky white mass of almost crystalline appearance.

For ease of reference the term σ-proteose will be used to describe the products thus obtained.

VII. *Properties of σ-proteose*

Judged by the nitrogen and phosphorus content, various batches of the alcohol-ether precipitated material appeared to be of uniform composition. More complete data for the composition of a typical preparation are given in Table 3 together with analyses of other milk proteins.

Table 3. *Percentage composition*

	σ-proteose* (moisture-free)	Casein(21)	Lactalbumin(20)	Lactoglobulin(20)	Palmer's(22) membrane protein
C	49·85	53·11	52·51	51·88	—
H	7·0	7·05	7·10	6·96	—
N	13·95	15·65	15·43	15·44	12·11–12·64
P	0·68	0·82	trace	0·24	0·27– 0·37
S	0·72	0·82	1·92	0·86	0·94– 0·96
Residue	3·2	—	—	—	—

The table shows that, apart from a markedly reduced N content, the composition of the σ-proteose resembles that of casein. The product contained no inorganic phosphorus; its calcium content amounted to 0·6%.

The freeze-dried σ-proteose disperses readily in water, the material obtained by alcohol-ether precipitation more slowly, dispersion being aided by warming and peptization with a little alkali. The sols thus obtained and the concentrates from which they are derived give positive biuret (bluish purple), Millon, and xanthoproteic reactions, and a negative Molisch test. They tend to foam copiously. The sols become increasingly turbid on acidification, and in concentrations exceeding about 50 mg.% a small amount of a fine precipitate settles out on standing at *p*H values between 4 and 5; on addition of further acid the turbidity disappears gradually with decreasing *p*H. Serial experiments with sols buffered to various *p*H values showed that precipitation occurred over a fairly wide range (4·8–4·1), being most pronounced at *p*H 4·3–4·7. Flocculation caused by addition of alcohol also showed a maximum at *p*H values between 4·3 and 4·7. The sols thus appear to be isoelectric at a point in the region of *p*H 4·5.

It will be remembered that the surface tension of milk sera obtained after removal of the casein and heat-coagulable proteins was found to be a minimum when the sera were adjusted to a *p*H of approximately 4·5; the same applied after such sera had been

* The author's thanks are due to Dr W. T. Chambers of the British Rubber Producers' Research Association for providing these micro-analytical results.

subjected to dialysis. It was found that this still holds after the σ-proteose has been concentrated and isolated as described above. Thus, it is observed throughout that the surface tension is at a minimum when the *p*H is adjusted to the point at which the fraction appears to be isoelectric. (Several authors have reported minimum values for σ at the isoelectric point of proteins (23–28), but others (29–31) observed the opposite. Further work clarifying these contradictory findings is clearly needed.) Fig. 6 shows that the procedures involved in concentrating and isolating the σ-proteose have not caused any marked loss in surface activity. (For stricter comparison the data are given on the basis of nitrogen content.) It is difficult to explain why the σ-dilution curves at a *p*H near 4·5 are practically

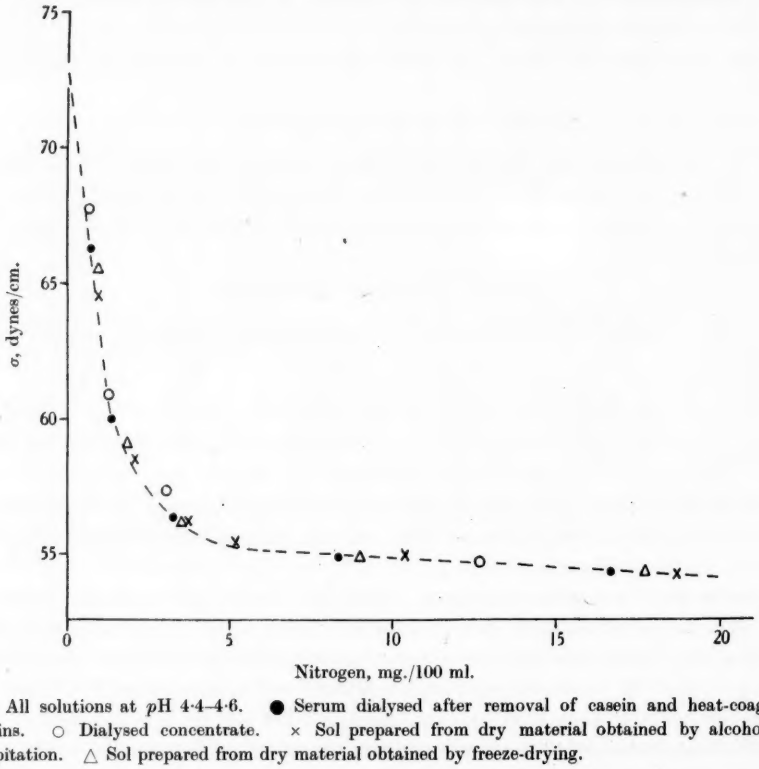

Fig. 6. All solutions at *p*H 4·4–4·6. ● Serum dialysed after removal of casein and heat-coagulable proteins. ○ Dialysed concentrate. × Sol prepared from dry material obtained by alcohol-ether precipitation. △ Sol prepared from dry material obtained by freeze-drying.

identical with the curves for the original milk samples. A further puzzling fact is the observation that the sols prepared from the dry material still show the characteristics of the original milk: curves, for instance, for preparations from another milk sample were very similar to those shown in Fig. 6, but ran on a higher σ-level in accordance with a somewhat higher surface tension of the original milk (56·3 dynes/cm.). One would have expected a loss of 'individuality' during the preparation of the dry material instead of the persistence of the characteristics determining the surface behaviour of the original milk samples. This persistence strengthens the suggestion that the type of surface-active material may vary to some extent from sample to sample, thus causing the variability in the σ-values of separated milk (see p. 318).

As the sols prepared from different batches of the dry material do not merely show a *random* variability in their surface behaviour (which, with colloids, might occur even with highly purified specimens), but a *systematic* connexion with the properties of the original milk, they are, clearly, not 'pure' in the strict sense of the word. Before attempting further purification it seemed desirable to get some information about the degree of homogeneity of the dry material. As the σ-proteose constitutes the major part of what is analytically termed the proteose-peptone fraction of milk,* it appeared possible that it might consist of a heterogeneous mixture of too complex a nature to attempt further fractionation.

Sols prepared from the freeze-dried specimen of σ-proteose (see p. 325) were subjected to electrophoretic and ultracentrifugal examination.

Dr R. A. Kekwick, whose kind help is gratefully acknowledged, 'examined a sol in phosphate buffer at *p*H 8, $\mu = 0{\cdot}2$, $\delta_n = 0{\cdot}00258$ in the Tiselius U-tube at 0° C., using the "diagonal Schlieren" optical method(32, 33). Three components were evident which in decreasing order of mobility accounted for 10·5, 82·5, and 7% respectively of the total protein. The main component gave a slightly asymmetrical peak.'

Whilst the bulk of the material thus proved to be electrophoretically homogeneous, the σ-proteose showed less homogeneity when examined in the ultracentrifuge (see Addendum). This apparent discrepancy is not uncommon (see, for instance, the recent work on a flavoprotein enzyme by Green *et al.*(34)). As pointed out by Shedlovsky(35), 'materials which lack homogeneity in the ultracentrifuge need not necessarily also do so in electrophoresis (since mobility depends primarily on charge distribution and not on size and mass), and vice versa'. The ultracentrifuge data show that the material, while far from homogeneous, contains a major, well-defined component ($S_{20} = 0{\cdot}96 \times 10^{-13}$) in sufficient amounts (49% of the total refracting substance) to encourage further work aimed at its isolation. It remains to be seen whether the surface activity of σ-proteose is due to this component which has a lower sedimentation constant—and probably a lower molecular weight—than the principal proteins of milk (see Svedberg(36)). The interesting foaming properties of σ-proteose, and of milk proteins in general, are under investigation.

SUMMARY

As moderate dilution causes little change in the surface tension of milk, it is shown to be advantageous to use σ-dilution curves in place of the σ-values of the undiluted fluid as a characteristic of the surface properties of milk. The complications arising from the presence of the milk fat are described, and it is suggested that the influence of the fat is of a physical rather than of a chemical nature. A study of the role of the various milk proteins shows the casein to be of great importance, whilst the heat-coagulable proteins have little influence. The serum obtained after removal of the casein and heat-coagulable proteins contains a residual fraction of protein-like material which is markedly surface active though constituting only about 3% of the total milk proteins. The surface-active material (σ-proteose) has been concentrated and isolated, and its properties are described in some detail.

* According to Rowland(17) the proteose-peptone N, i.e. the residual N content other than non-protein N in milk from which the casein and heat-coagulable proteins have been removed, amounts to *c.* 4% of the total N. As shown on p. 324, such milk contains *c.* 3% of the total N in a non-dialysable form.

ADDENDUM: EXAMINATION IN THE ULTRACENTRIFUGE

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The preparation was examined in the Svedberg oil-turbine ultracentrifuge by the 'diagonal schlieren' method of Philpot(32). The dried material was dissolved to 1·35% in 0·2*M* sodium chloride, 0·017*M* Na_2HPO_4 and 0·017*M* KH_2PO_4; *p*H 6·8. The solution was only slightly opalescent. The speed of rotation was 1020 rev./sec.

There appeared to be two distinct components of $S_{20} = 0{\cdot}96 \times 10^{-13}$ and $2{\cdot}75 \times 10^{-13}$; both appeared to be homogeneous. They account for 49 and 11% of the total refractive material respectively. The remaining 40% does not appear in the sedimentation diagrams; this may mean that it is too highly polydisperse to form an observable boundary, or that it is of molecular weight too low to sediment noticeably, or that it sedimented out completely before observations could be made. The minimum molecular weights of the two components are 4900 and 23,900, assuming that the molecules are spherical and that the partial specific volumes are 0·75. The boundary due to the lighter component appears to be rather less diffuse than would be expected from so low a molecular weight, so that it is likely that the molecular weight is somewhat greater than the minimum and the molecule correspondingly aspherical (Fig. 7).

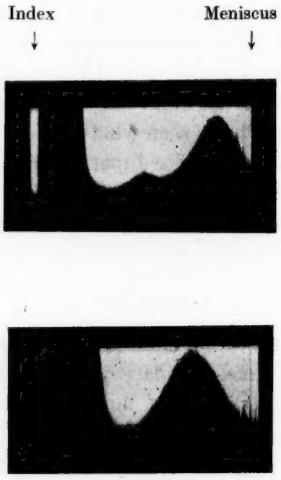

Fig. 7.

Fig. 7 shows the sedimentation diagram 2 hr. 20 min. and 4 hr. 40 min. after reaching full speed; in the latter the heavier component has reached the bottom of the cell.

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337. THE SUPPLEMENTARY RELATIONSHIPS BETWEEN THE PROTEINS OF DAIRY PRODUCTS AND THOSE OF BREAD AND POTATO AS AFFECTED BY THE METHOD OF FEEDING.* WITH A NOTE ON THE VALUE OF SOYA-BEAN PROTEIN

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It is well known that the proteins of milk exert a supplementary effect on those of cereals and other foods(2, 3, 4, 5, 6, 7, 8, 9), but little seems to be known about the value in this respect of cheese and of whey. In the present work the supplementary effects were studied of the proteins of milk, cheese and whey on those of bread and of potato.

Some time ago when examining by the method of Mitchell(7, 10) the biological value of the proteins of milk, we pointed out(11) that, when milk was fed separately from the basal 'nitrogen-free' diet, lower biological values were obtained for it than when it was incorporated in the diet. Subsequent work(12) lent support to this suggestion, but in experiments designed to put the matter to direct test(13) the results, although suggestive, lacked statistical significance. Berg & Rose(14) found that rats receiving a tryptophane-deficient diet grew better when given 10 mg. of tryptophane every 12 hr. than with a supplement of 40 mg. every 48 hr.; in either case the growth was inferior to that observed when the same proportion of the amino acid was incorporated in the diet. More recently, Elman(15) obtained similar results with dogs, while Cuthbertson and his colleagues(16, 17) found that when the protein and carbohydrate fractions of a diet, jointly capable of maintaining nitrogen equilibrium, were given at different times of the day to human adults(16) or adult rats(17) the nitrogen balance became negative and there was loss of body weight.

All these findings emphasize the importance of time and concentration relationships in the utilization of dietary protein. Further information was sought in the experiments described in this paper.

EXPERIMENTAL

Separate experiments were carried out to determine the supplementary relationships between the proteins of (1) bread and cheese, (2) potato and dried skim milk, (3) potato and dried whey. In the first two experiments the effect of offering the two proteins together or separately on alternate days was studied. A test of the biological value of the proteins of soya-bean flour was included in the third experiment, as this information was wanted at the time in view of the importance of this foodstuff in war-time dietaries. In all experiments the biological value and true digestibility of the proteins were determined by the method of Mitchell(7, 10). A full description of the experimental technique has been given elsewhere(11). The level of protein intake ($N \times 6{\cdot}38$ for milk, cheese and whey and $N \times 6{\cdot}25$ for the other substances) was 8·5% in Exp. 1, and 8·0% in Exps. 2 and 3.

* Read in part before the Biochemical Society, February 1942 (Henry & Kon(1)).

Exp. 1. *The supplementary relationship between proteins of white bread and of Cheddar cheese.*

Dried white bread tested by us in earlier experiments(18) was used; the Cheddar cheese was made at this Institute and was vacuum-ice dried at the Low Temperature Research Station, Cambridge. The composition and analysis of the experimental diets are shown in Table 1. Diet 152 was the bread diet. Sufficient margarine fat was included to bring its fat content to that of the cheese diet (diet 153). Diet 154 consisted of equal parts of diets 152 and 153. Three groups of four litter-mate female rats 24–28 days old and weighing 42–80 g. were used. In the course of the experiment each animal received in turn the following diets: diet 152, diet 153, diet 154, and finally, on alternate days, equal amounts of diets 152 and 153, the quantities being adjusted every 2 days according to appetite. The proportion of bread to cheese as fed in diet 154 was 2·8 parts of dried bread to 1 part of dried cheese, corresponding approximately to 3·5 parts of bread to 1 part of cheese as normally consumed.

Results.

The results of this experiment are given in Tables 2–4. The biological value of 52 now obtained for white bread was rather higher than the values of 45 and 48 previously obtained by us with the same bread(18), while the value of 76 obtained for the cheese was slightly higher than the value of 72 previously found in this laboratory(19) for a different sample of Cheddar cheese, and of 73 observed by Beadles, Quisenberry, Nakamura & Mitchell(20) for Swiss cheese, both at an 8% level of protein. With the bread and cheese fed together, each supplying 50% of the protein, a marked supplementary effect was obvious as the biological value of the mixture, 75, was almost identical with that of the cheese alone. When, however, the two proteins were fed on alternate days the resulting biological value of 67 was only slightly higher than the value of 64 which would be expected if there were no supplementary relationship. The values found now for the true digestibility of bread, 99, and of Cheddar cheese, 100, were higher than the values 93 and 98, respectively, previously found in this laboratory for these foods(18, 19). The true digestibility of the mixture of proteins (diet 154) was 98 and of the diets fed alternately, 99.

Exp. 2. *The supplementary relationship between the proteins of potato and of dried skim milk.*

'Majestic' potatoes were used. They were boiled in their skins, peeled, sieved and dried in a hot-air oven at 110–140° F. The milk was a high-quality spray-dried skim product. The composition and analysis of the experimental diets are given in Table 1. The proportion of milk to potato as fed in the diet was 1 : 3·5 or approximately 1 part liquid milk to 1·3 parts of boiled potato. Three litters of four female rats 24–26 days old and weighing 54–62 g. were used and, as in the previous experiment, received in turn diets 163 (potato), 164 (milk), 165 (potato + milk) and, on alternate days, equalized amounts of diets 163 and 164.

Results.

The results are given in Tables 3–5. The biological value of 81 obtained when the proteins were fed separately was almost identical with the value of 80 which would be expected if no supplementary relationship existed between these two proteins. When

the milk and potato were given together a value of 86 was obtained, indicating a definite supplementary effect. Table 4 shows that, as in Exp. 1, the differences observed when the two proteins were fed together or separately were statistically significant. The biological value of 89 for the dried milk falls within the range of 80–90 previously observed by us for raw liquid (11, 12, 21) and for dried milks (13, 22). The value of 71 obtained for potato is in line with values of 67–69 (23) and of 71 for the potato globulin, tuberin (24).

The true digestibility of the proteins was 82 for potato and 90 for milk (Table 3). When the two proteins were fed together the true digestibility amounted to 82, a value equal to that of potato alone. The digestibility was 87 for milk and potato fed on alternate days, a figure very close to the mean of the separate values for the two. The difference between the values of 82 and 87 is statistically significant (Table 4).

The results of these two experiments show very clearly that in order that two proteins may exert a supplementary effect on each other they should be given together. This is not surprising when the mechanism of the effect (25) is considered. It is unlikely that the surplus amino acids of the one food would be stored by the animal until the 'supplementary' acids from the other are fed on the following day.

Exp. 3. *The lack of supplementary relationship between the proteins of potato and of dried whey.*

This experiment was carried out with another sample of 'Majestic' potatoes prepared as in Exp. 2 and with a sample of high-quality spray-dried whey. This time it was planned to feed the potato diet (diet 167), the whey diet (diet 168) and a diet (diet 169) consisting of 3 parts diet 167 and 1 part diet 168. In this way the whey supplied 25% of the protein instead of 50% supplied by milk or cheese in Exps. 1 and 2; this level was thought to be high enough for a supplementary effect to become evident, if it existed, and yet nearer practical possibilities than the 50% level.

A test was included with a sample of proprietary edible soya-bean flour (diet 170). This type of flour is prepared by treating soya beans with steam to remove the 'beany' taste of the raw product and to prevent development of rancidity. The composition and analysis of these diets is given in Table 1. Three litters of four female rats 25 days old and weighing 50–60 g. were used. The diets were tested in turn on each rat.

Results.

The results are shown in Tables 3, 4 and 6. Unfortunately, in the second experimental period, three rats receiving the whey diet lost weight and died. In the first experimental period they had received diet 169, i.e. the mixture of potato and whey, and it seems likely that they succumbed to the cumulative effects of the high lactose content of the diets (26). For the remainder of the experiment the feeding of the whey diet was discontinued.

In consequence, figures for the biological value of the proteins of whey are available from only three rats and are respectively 77, 22 and 85. The figure for the second rat is obviously abnormal, but there was no apparent reason for rejecting it. It is probably indicative of the difficulties caused by the high lactose content of the diet. The other two values are not far removed from Kik's (27) estimate of 84 for the biological value of lactalbumin, the chief source of nitrogen in whey. On the assumption that the biological value of the whey protein is of the same order as that of lactalbumin our results show no indication of a supplementary relationship between whey and potato proteins. In fact, the value for the mixture of the two foods, 70, is lower than 76 obtained for potato alone. The rats receiving this mixture behaved apparently normally, but the scatter of individual

results was high, values ranging from 53 to 88 being obtained, and it is possible that the disturbing effects of the whey diet were also to a certain extent manifested by the mixture.

During the last experimental period the six rats which had not received the whey diet were given again the diet they had had during the first experimental period. These results are not included in the averages or used in the statistical analysis.

The true digestibility of the proteins of the potato and whey diet was 68, a value significantly lower than that of 82 for potato. The three rats on which the whey diet was tested yielded values of 56, 72 and 63 with a mean of 64. In this experiment the findings with whey and potato confirm those obtained with milk and potato to the effect that the true digestibility of a mixture of two proteins is similar to that of the less digestible component. Further work has shown, however, that this is not necessarily always the case (31).

The sample of soya flour tested had a biological value of 73 and a true digestibility of 90. The discussion of these values will be postponed until further work with soya bean now in progress is completed.

Table 1. *Composition and analysis of the experimental diets*

	Exp. 1			Exp. 2			Exp. 3			
Component	Diet 152	Diet 153	Diet 154	Diet 163	Diet 164	Diet 165	Diet 167	Diet 168	Diet 169	Diet 170
Rice starch	11·7	51·4	31·55	—	41·5	20·75	2·3	12·4	4·8	56·45
Potato starch	10·0	10·0	10·0	7·0	10·0	8·5	10·0	10·0	10·0	10·0
Sugar (granulated, ground)	—	12·0	6·0	—	12·0	6·0	—	—	—	—
Margarine fat	11·0	—	5·5	10·0	10·0	10·0	10·0	10·0	10·0	10·0
Salts (de Loureiro (28))	4·0	4·0	4·0	4·0	4·0	4·0	4·0	4·0	4·0	4·0
White bread (dried)	63·3	—	31·65	—	—	—	—	—	—	—
Cheddar cheese (dried)	—	22·6	11·3	—	—	—	—	—	—	—
Potato (cooked, dried)	—	—	—	79·0	—	39·5	73·7	—	55·3	—
Dried skim milk	—	—	—	—	22·5	11·25	—	—	—	—
Dried whey	—	—	—	—	—	—	—	63·6	15·9	—
Soya flour	—	—	—	—	—	—	—	—	—	19·55
% Nitrogen	1·391	1·329	1·385	1·285	1·262	1·269	1·297	1·245	1·268	1·298
% Moisture	8·87	10·50	9·83	5·30	9·54	7·35	8·29	12·95	8·83	10·99

Table 2. *Individual nitrogen metabolism data for 6-day periods. Exp.* 1

Rat no.	Diet* tested	Weight of rat (g.) Initial	Final	Diet† intake (g.)	N intake (mg.)	Faecal N (mg.)	Urinary N (mg.)	Biological value	True digestibility
				Low egg-nitrogen diet					
1	—	69	71	45·65	—	101·6	92·7	—	—
2	—	76	81	55·09	—	105·6	103·7	—	—
3	—	73	73	38·46	—	80·9	89·1	—	—
4	—	72	74	49·76	—	117·0	93·9	—	—
5	—	50	54	43·12	—	82·5	65·9	—	—
6	—	45	49	39·29	—	68·5	69·8	—	—
7	—	46	50	36·74	—	67·3	65·9	—	—
8	—	47	52	47·45	—	85·4	74·9	—	—
9	—	52	59	48·59	—	87·9	75·0	—	—
10	—	50	54	41·83	—	84·5	68·6	—	—
11	—	50	57	42·63	—	76·3	66·2	—	—
12	—	48	55	42·12	—	83·1	64·5	—	—
				First experimental period					
1	A	76	81	50·56	703·3	72·4	—	—	100·0
2	B	88	104	60·99	810·6	127·0	272·2	80·7	99·9
3	C	78	84	41·81	578·8	107·6	250·6	72·0	89·6
4	D	79	83	37·97	516·4	89·9	240·3	69·0	100·0
5	A	56	57	36·19	503·4	95·3	311·8	48·9	99·0
6	B	57	61	33·36	443·4	73·1	217·2	68·2	99·4
7	C	54	66	40·96	566·9	87·7	189·8	80·0	99·7
8	D	57	62	39·00	530·4	94·3	245·0	68·2	99·2
9	A	58	61	36·62	509·3	98·0	321·1	48·6	98·8
10	B	58	69	40·49	538·3	84·4	205·2	76·1	99·9
11	C	65	79	50·99	705·8	126·7	238·9	76·7	99·2
12	D	56	58	28·63	388·9	63·4	193·6	66·6	99·7

Table 2 (*continued*)

Rat no.	Diet* tested	Weight of rat (g.) Initial	Final	Diet† intake (g.)	N intake (mg.)	Faecal N (mg.)	Urinary N (mg.)	Biological value	True digestibility
				Second experimental period					
1	B	92	116	70·84	941·4	176·6	248·4	75·2	99·5
2	C	113	128	64·49	892·8	174·9	304·9	80·2	99·2
3	D	89	100	50·70	689·5	141·2	297·2	70·6	99·2
4	A	88	98	56·74	789·3	165·5	438·3	54·0	99·2
5	B	64	81	54·57	725·4	137·0	183·7	85·0	99·2
6	C	65	73	38·99	539·6	79·7	248·5	69·3	99·6
7	D	67	80	47·86	651·1	119·9	276·8	70·0	99·3
8	A	68	76	55·05	765·8	167·3	405·3	55·5	98·5
9	B	67	84	51·29	681·9	117·8	197·1	83·2	99·4
10	C	80	97	58·97	816·2	153·7	253·9	79·8	99·3
11	D	83	92	54·15	736·7	129·5	292·1	72·2	99·5
12	A	64	68	37·16	516·9	113·5	315·9	48·7	98·5
				Third experimental period					
1	C	125	145	81·34	1126·0	235·1	425·8	73·7	99·0
2	D	131	139	59·71	812·1	182·8	393·3	66·6	98·8
3	A	107	117	63·45	882·4	188·2	524·9	51·0	99·1
4	B	108	130	73·87	981·9	172·1	361·5	74·5	100·0
5	C	92	107	66·28	917·5	194·0	302·3	76·9	98·9
6	D	79	91	50·11	681·4	119·8	315·6	66·3	99·2
7	A	85	89	43·83	609·6	115·3	378·4	51·8	99·2
8	B	82	103	66·00	877·2	155·3	302·5	77·1	99·6
9	C	92	114	71·08	984·0	190·0	316·3	77·7	99·0
10	D	100	111	54·91	747·0	167·0	406·1	56·2	98·6
11	A	96	108	66·60	926·4	178·3	471·0	58·2	99·3
12	B	73	85	46·50	618·1	109·5	305·8	62·1	99·6
				Fourth experimental period					
1	D	145	143	47·93	651·9	146·2	395·7	58·5	98·8
2	A	145	150	58·87	818·9	185·6	479·9	55·6	98·9
3	B	124	134	59·71	793·8	152·4	324·7	74·1	99·7
4	C	138	152	72·96	1010·0	213·4	391·8	73·1	99·3
5	D	115	120	55·91	760·4	159·2	332·7	68·9	98·8
6	A	96	98	45·22	629·0	116·1	407·0	48·1	98·9
7	B	95	107	47·88	636·3	120·3	236·4	78·7	99·4
8	C	116	125	65·27	903·5	185·8	335·9	75·3	99·0
9	D	123	129	61·89	841·8	196·6	391·8	64·6	98·3
10	A	117	131	69·56	967·7	232·3	530·6	53·6	98·2
11	B	115	133	71·00	943·9	154·9	395·6	71·5	100·0
12	C	94	104	49·42	684·1	128·6	291·9	69·0	99·3
				Low egg-nitrogen diet					
1	—	146	156	61·49	—	133·5	136·1	—	—
2	—	152	152	51·33	—	127·1	125·5	—	—
3	—	139	142	55·66	—	135·3	123·7	—	—
4	—	156	163	68·38	—	164·4	139·8	—	—
5	—	126	133	60·45	—	120·6	111·7	—	—
6	—	97	102	38·88	—	71·3	89·2	—	—
7	—	108	106	53·96	—	119·3	101·9	—	—
8	—	126	133	69·39	—	153·8	118·1	—	—
9	—	134	146	68·96	—	140·4	119·9	—	—
10	—	132	138	57·87	—	123·6	97·9	—	—
11	—	144	154	69·98	—	167·9	117·9	—	—
12	—	112	128	66·70	—	140·7	94·9	—	—

* A = diet 152; B = diet 153; C = diet 154; D = diets 152 and 153 in equal amounts on alternate days.

† As the rats receiving diet D were offered, and consumed, almost identical quantities of diets 152 and 153 only the total consumption is quoted here to economize space.

Table 3. *Mean biological values and true digestibilities of the foods tested in the three experiments*

	Biological value				
	Period 1	Period 2	Period 3	Period 4	Mean
Exp. 1: White bread (dried)	48·8*	52·7	53·7	52·4	52·2
Cheddar cheese	75·0	81·1	71·2	74·8	75·5
Bread and cheese:					
Fed together	76·2	76·4	76·1	72·5	75·3
Fed separately on alternate days	67·9	70·9	63·0	64·0	66·5
Exp. 2: Potato (boiled and dried)	78·8	71·2	66·9	68·8	71·4
Dried skim milk	95·5	90·3	86·1	82·3	88·5
Potato and milk:					
Fed together	91·3	87·8	87·4	76·4	85·7
Fed separately on alternate days	86·6	81·6	77·4	76·6	80·5
Exp. 3: Potato (boiled and dried)	75·4	75·0	78·0	(69·1)	76·1
Dried whey	61·1	—	—	—	61·1
Potato and whey, fed together	(81·8)	72·8	70·6	67·9	70·4
Soya flour	74·0	70·9	74·6	(65·4)	73·2

	True digestibility				
	Period 1	Period 2	Period 3	Period 4	Mean
Exp. 1: White bread (dried)	99·3	98·7	99·2	98·7	99·0
Cheddar cheese	99·7	99·4	99·7	99·7	99·6
Bread and cheese:					
Fed together	96·2	99·4	99·0	99·2	98·4
Fed separately on alternate days	99·6	99·3	98·9	98·6	99·1
Exp. 2: Potato (boiled and dried)	86·3	82·5	82·6	77·5	82·2
Dried skim milk	92·9	89·3	88·4	88·5	89·8
Potato and milk:					
Fed together	84·5	80·8	81·1	83·2	82·4
Fed separately on alternate days	88·0	86·2	87·1	86·2	86·9
Exp. 3: Potato (boiled and dried)	80·7	80·2	84·6	(81·1)	81·8
Dried whey	63·8	—	—	—	63·8
Potato and whey, fed together	(74·5)	68·1	68·1	67·0	67·7
Soya flour	91·7	89·1	90·5	(86·6)	90·4

* For two rats only, urinary analysis lost for third rat.

Figures in parentheses are not included in the mean values, cf. p. 332.

Table 4. *Statistical analysis* of some of the differences observed in the three experiments*

Difference tested	Numerical difference	Standard error of the mean	P†
Exp. 1. Bread and cheese			
Biological value: Fed together – fed separately	+ 9·0	±1·9	1 : 1667 Significant
Exp. 2. Potato and milk			
Biological value: Fed together – fed separately	+ 5·2	±2·0	1 : 38 Significant
True digestibility: Fed together – fed separately	− 4·5	±1·1	1 : 556 Significant
Exp. 3. Potato and whey			
Biological value: Potato – Potato + whey	+ 5·8	±3·7	1 : 6 Not significant
True digestibility: Potato – Potato + whey	+14·1	±2·0	1 : 8930 Significant

* The paired t-test of 'Student' (29, 30) was used.

† P = probability that a mean difference at least as great as the observed mean difference would have arisen by random sampling from a homogeneous population.

Table 5. *Individual nitrogen metabolism data for 6-day periods. Exp.* 2

Rat no.	Diet* tested	Weight of rat (g.) Initial	Final	Diet† intake (g.)	N intake (mg.)	Faecal N (mg.)	Urinary N (mg.)	Biological value	True digestibility
				Low egg-nitrogen diet					
13	—	58	67	55·28	—	106·9	79·2	—	—
14	—	60	68	54·00	—	114·4	78·3	—	—
15	—	59	67	55·03	—	97·2	81·9	—	—
16	—	59	60	40·88	—	82·9	81·4	—	—
17	—	57	61	43·11	—	86·3	73·5	—	—
18	—	57	60	46·84	—	108·5	70·3	—	—
19	—	60	64	47·14	—	90·6	73·4	—	—
20	—	52	56	41·97	—	93·1	71·5	—	—
21	—	58	64	51·89	—	105·5	70·8	—	—
22	—	54	60	47·92	—	101·7	70·9	—	—
23	—	58	62	45·74	—	104·0	68·5	—	—
24	—	59	60	43·85	—	94·4	71·1	—	—
				First experimental period					
13	E	68	78	46·58	598·5	199·6	201·5	77·0	82·3
14	F	74	92	55·48	700·1	184·3	124·0	95·7	90·8
15	G	77	89	57·98	735·8	234·0	173·8	88·4	83·0
16	H	66	76	44·99	573·0	205·0	146·1	88·1	80·6
17	E	68	80	48·97	629·4	188·2	199·9	79·6	86·2
18	F	67	85	55·46	700·0	157·7	127·2	93·9	95·8
19	G	74	93	60·80	771·6	237·0	141·9	92·6	84·9
20	H	63	72	41·90	533·5	146·0	158·0	84·7	89·9
21	E	66	78	49·50	636·0	162·2	196·2	79·7	90·4
22	F	69	89	61·43	775·4	192·9	115·3	96·8	92·2
23	G	68	85	55·33	702·1	224·9	125·2	92·9	85·5
24	H	64	78	44·99	573·0	135·1	148·7	87·1	93·5
				Second experimental period					
13	H	80	85	45·74	582·4	180·2	204·6	78·2	85·6
14	E	100	111	67·50	867·4	318·1	270·2	78·1	80·8
15	F	102	120	75·82	957·0	270·5	187·1	93·4	87·5
16	G	84	98	65·76	834·7	324·8	194·5	87·0	77·8
17	H	85	97	60·13	765·9	252·7	193·2	86·4	83·8
18	E	94	90	45·67	586·9	204·8	276·7	63·7	83·1
19	F	99	119	72·96	920·8	243·9	206·8	88·7	89·6
20	G	81	96	61·97	786·5	266·2	182·7	87·8	83·2
21	H	87	101	64·97	827·5	225·0	245·3	80·1	89·1
22	E	89	99	60·97	783·4	262·1	287·3	71·9	83·5
23	F	93	106	65·87	831·4	220·7	186·8	88·5	90·7
24	G	87	105	66·75	847·0	302·7	180·4	88·6	81·5
				Third experimental period					
13	G	93	103	57·31	727·4	257·2	180·2	88·0	81·8
14	H	112	123	62·99	802·2	251·1	272·6	78·5	86·8
15	E	124	132	76·87	988·9	342·2	392·7	69·3	81·8
16	F	110	126	74·49	940·1	295·2	269·9	83·2	85·8
17	G	106	118	68·98	875·4	315·2	198·8	89·7	81·4
18	H	91	94	40·72	518·6	148·9	226·6	71·7	89·4
19	E	121	119	52·98	681·0	219·6	306·3	66·8	84·1
20	F	102	113	63·46	800·9	202·0	217·8	86·2	91·8
21	G	107	123	76·50	970·9	354·6	235·6	84·4	80·0
22	H	109	121	70·98	903·9	291·9	256·8	81·9	85·1
23	E	108	112	51·92	667·3	231·8	296·0	64·5	81·8
24	F	114	128	73·94	933·0	277·9	209·6	89·0	87·7

Table 5 (*continued*)

Rat no.	Diet* tested	Weight of rat (g.) Initial	Final	Diet† intake (g.)	N intake (mg.)	Faecal N (mg.)	Urinary N (mg.)	Biological value	True digestibility
				Fourth experimental period					
13	F	111	124	66·98	845·3	288·5	245·7	83·0	83·8
14	G	130	142	72·97	926·0	361·9	277·6	80·5	79·5
15	H	133	143	70·93	903·3	256·1	317·1	78·9	89·0
16	E	130	136	67·99	837·8	359·9	344·9	67·7	72·0
17	F	128	140	74·98	946·4	259·5	280·9	84·1	90·7
18	G	99	105	45·35	575·5	177·5	243·2	71·3	87·3
19	H	123	132	59·97	763·8	236·6	278·7	75·0	85·8
20	E	119	123	58·99	758·0	261·9	314·1	68·7	81·9
21	F	135	147	75·79	956·6	254·8	307·2	79·9	90·9
22	G	132	140	70·94	900·1	312·4	299·2	77·3	82·9
23	H	116	124	59·97	763·8	247·4	258·3	75·8	83·9
24	E	131	139	70·99	912·2	353·4	340·7	70·0	78·6
				Low egg-nitrogen period					
13	—	127	139	75·98	—	175·9	134·4	—	—
14	—	144	156	74·99	—	182·0	138·5	—	—
15	—	144	155	74·95	—	173·9	152·0	—	—
16	—	137	149	72·29	—	166·2	141·9	—	—
17	—	142	146	66·13	—	155·2	148·9	—	—
18	—	107	113	47·48	—	108·8	100·9	—	—
19	—	137	143	59·31	—	130·5	115·2	—	—
20	—	124	138	73·71	—	155·0	119·3	—	—
21	—	149	152	62·15	—	133·0	132·8	—	—
22	—	142	152	74·43	—	169·3	129·4	—	—
23	—	131	144	74·96	—	151·2	109·5	—	—
24	—	139	149	74·17	—	166·3	124·3	—	—

* E = diet 163; F = diet 164; G = diet 165; H = diets 163 and 164 in equal amounts on alternate days.

† As the rats receiving diet H were offered, and consumed, almost identical quantities of diets 163 and 164, only the total consumption is quoted here to economize space.

Table 6. *Individual nitrogen metabolism data for 6-day periods. Exp.* 3

Rat no.	Diet* tested	Weight of rat (g.) Initial	Final	Diet intake (g.)	N intake (mg.)	Faecal N (mg.)	Urinary N (mg.)	Biological value	True digestibility
				Low egg-nitrogen diet					
25	—	58	65	43·73	—	77·9	82·4	—	—
26	—	57	63	43·17	—	90·2	75·7	—	—
27	—	57	59	36·37	—	77·1	78·1	—	—
28	—	58	61	38·24	—	94·5	73·4	—	—
29	—	58	61	37·03	—	75·6	81·4	—	—
30	—	59	63	39·82	—	79·6	76·2	—	—
31	—	58	57	22·57	—	39·7	73·6	—	—
32	—	57	56	26·24	—	49·5	71·7	—	—
33	—	53	53	31·89	—	79·8	68·7	—	—
34	—	50	54	33·77	—	77·5	66·9	—	—
35	—	51	54	34·76	—	82·0	68·3	—	—
36	—	51	55	37·82	—	86·0	69·4	—	—
				First experimental period					
25	J	69	77	48·50	629·1	224·2	218·8	74·1	78·6
26	K	61	64	31·70	394·7	237·7	128·6	76·7	56·3
27	L	63	72	41·49	526·0	221·1	138·7	88·0	74·7
28	M	65	73	43·48	564·4	145·0	191·2	78·7	92·5
29	J	67	75	50·48	654·7	238·3	215·4	76·0	79·4
30	K	59	60	27·51	342·6	153·4	264·5	21·6	71·6
31	L	60	66	38·92	493·4	193·2	182·3	72·3	74·7
32	M	61	67	37·16	482·4	118·0	223·2	66·7	90·3
33	J	57	65	45·98	596·5	204·8	196·1	76·1	84·2
34	K	53	57	30·95	385·4	212·0	108·3	85·0	63·4
35	L	59	67	43·08	546·3	242·5	141·3	85·2	74·2
36	M	59	71	50·47	655·1	164·8	224·1	76·6	92·3

Table 6 (*continued*)

Rat no.	Diet* tested	Weight of rat (g.) Initial	Final	Diet intake (g.)	N intake (mg.)	Faecal N (mg.)	Urinary N (mg.)	Biological value	True digestibility
				Second experimental period					
25	M	81	94	58·01	753·0	225·9	266·2	74·3	84·8
26	J	69	82	54·00	700·5	261·5	196·7	80·4	78·3
28	L	78	87	45·49	576·9	271·0	146·2	86·6	70·8
29	M	79	89	56·96	739·4	204·0	316·3	67·0	88·3
30	J	60	72	47·00	609·7	212·3	235·7	67·8	81·1
32	L	70	81	43·63	553·2	227·4	178·9	78·3	74·4
33	M	69	77	47·02	610·4	145·1	250·6	71·5	94·1
34	J	63	74	54·00	700·5	255·1	222·6	76·9	81·2
36	L	74	78	34·37	435·8	256·8	212·6	53·4	59·0
				Third experimental period					
25	L	94	96	45·97	582·9	281·3	235·7	65·9	67·4
26	M	84	95	54·50	707·5	196·6	240·5	77·7	87·6
28	J	87	97	53·26	690·9	230·2	225·9	77·4	83·2
29	L	95	94	41·00	519·8	239·8	222·5	68·3	70·2
30	M	76	90	58·49	759·3	189·9	272·8	73·9	91·1
32	J	79	94	58·49	758·7	231·8	256·5	75·5	84·7
33	L	78	80	46·53	590·0	300·0	178·5	77·5	66·7
34	M	80	93	60·22	781·6	194·8	317·5	72·2	92·8
36	J	77	90	57·00	739·5	235·4	218·1	81·2	85·8
				Fourth experimental period					
25	J	93	102	55·50	720·0	236·7	303·5	65·4	83·0
26	L	102	97	46·93	595·1	335·5	185·7	78·2	59·2
28	M	96	104	55·50	720·4	190·3	326·5	64·5	89·2
29	J	91	99	54·00	700·5	232·5	273·1	70·1	82·9
30	L	91	91	45·46	576·5	273·2	236·4	65·2	69·4
32	M	99	103	51·40	667·3	239·5	327·5	59·2	79·6
33	J	79	87	56·99	739·2	288·1	250·8	71·9	77·5
34	L	84	82	36·16	458·4	209·4	243·7	60·4	72·5
36	M	94	104	64·78	840·9	223·5	323·0	72·4	91·0
				Low egg-nitrogen diet					
25	—	110	118	59·62	—	128·3	102·3	—	—
26	—	100	112	59·49	—	115·7	111·0	—	—
28	—	113	124	65·50	—	125·0	109·6	—	—
29	—	104	114	63·00	—	131·9	106·2	—	—
30	—	92	99	53·59	—	114·4	98·4	—	—
32	—	109	118	58·37	—	118·7	120·8	—	—
33	—	94	104	62·68	—	128·5	103·1	—	—
34	—	84	97	60·75	—	139·5	124·3	—	—
36	—	107	114	63·79	—	145·2	120·5	—	—

* J = diet 167; K = diet 168; L = diet 169; M = diet 170.

Summary

1. The biological value of proteins at an 8% level of protein intake measured on rats by the method of Mitchell (7, 10) was 52 for white bread of 73% extraction, 76 for Cheddar cheese, 75 for a mixture of both foods when each supplied 50% of the protein, and 67 when the two sources of protein were given on alternate days. The true digestibilities were, respectively, 99, 100, 98 and 99.

2. Similar experiments with dried skim milk and dried cooked potatoes yielded biological values of 71 for potato, 89 for milk, 86 for the mixture and 81 for the substances fed separately on alternate days. The true digestibilities were, respectively, 82, 90, 82 and 87.

3. An attempt to carry out experiments of the same type with dried whey and dried

potato failed because of deaths of some rats receiving the whey diet, probably because of its high content of lactose. A biological value of 76 was obtained for the potato proteins and of 70 for a mixture of whey and potato in which whey supplied 25% of the protein. The true digestibilities were, respectively, 82 and 68.

4. The biological value and the true digestibility of the proteins of a sample of edible soya-bean flour were, respectively, 73 and 90.

5. It is concluded that there is a marked supplementary relationship between the proteins of milk and potato and those of bread and cheese when the members of each pair supply equal amounts of protein and when they are given together. No supplementary relationship is exhibited when the sources of protein are given separately on alternate days.

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543.85:637.0

338. THE FERRIC THIOCYANATE METHOD OF ESTIMATING PEROXIDE IN THE FAT OF BUTTER, MILK AND DRIED MILK

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(With 2 Figures)

Introduction

In butter, milk, and dried milk, flavour deterioration accompanies a slight degree of oxidation of the butterfat, and for the study of oxidative deterioration of these products a method is required which measures fat oxidation at a very early stage. In this laboratory it has been found that the iodometric peroxide method of Lea (1), although slightly more sensitive than the fat aldehyde test of Schibsted (2), registers measurable values only at about the stage of oxidation of butterfat when flavour changes first appear. The work of Chapman & McFarlane (3) and of Lips *et al.* (4) has directed attention to the value of the ferric thiocyanate peroxide test in the study of oxidation of the fat in dairy products, and it was hoped to use this method in studying oxidation before flavour changes could be detected. Difficulties were encountered in the low solubility of fat in 96% acetone, and in the rapid oxidation of ferrous salts in this solvent. Modifications have been made to the test, and in its final form it has proved sufficiently sensitive to follow the oxidation of the fat of milk from the time it leaves the udder.

Method

Reagents

(1) *Benzene-methanol*

The solvent is a mixture of 70 vol. of benzene and 30 vol. of methanol. A.R. quality benzene is redistilled and the first three 20 ml. lots are used to rinse the receiving vessel. Synthetic methanol is dried by refluxing during 4 hr. with bright magnesium ribbon (5 g./l.), and is then distilled. The moisture content of the mixed solvent should not exceed 0·5%. This may be checked with the Karl Fischer reagent (5).

As the benzene-methanol mixture is cheap, its recovery is probably not economical. When necessary it may be recovered by adjusting the acidity with a saturated solution of potassium hydroxide in methanol to give a reading of *p*H 7 with the glass electrode (to avoid distillation of ammonia or thiocyanic acid), refluxing during 4–5 hr. with 20-mesh anhydrous calcium sulphate, and then distilling. The moisture content of the recovered reagent should be checked and the *p*H value determined and adjusted if necessary to between 6·0 and 7·0. The proportion of benzene and methanol may be corrected by adjusting the specific gravity to 0·850 at 20° C.

(2) *Ferrous chloride solution*

Hydrated barium chloride (0·4 g.) dissolved in 50 ml. water is added slowly, with stirring, to 0·5 g. hydrated ferrous sulphate dissolved in 50 ml. water, and 2 ml. of 10*N* hydrochloric acid is then added. All reagents should be free of ferric iron. The precipitate

of barium sulphate is allowed to settle, and the clear solution is then decanted into a bottle protected from light. The solution is sufficiently stable to be usable for about a week; the extent of oxidation may be judged by noting the colour obtained when 1 drop of 30% ammonium thiocyanate is added to 1 ml. of the ferrous chloride solution. A solution containing one-fifth this concentration of ferrous salt, but the same concentration of hydrochloric acid, should be used for peroxide values below 0·1.

(3) *Ammonium thiocyanate solution*

The solution is made by dissolving 30 g. ammonium thiocyanate in water and adjusting the volume to 100 ml. Potassium thiocyanate is not suitable. For convenience of handling the ammonium thiocyanate and ferrous chloride solutions a 1 ml. graduated pipette is left in each reagent bottle. The pipette is fitted with a glass hood consisting of a Gooch crucible holder which fits over the neck of the bottle.

(4) *Artificial standard*

This is the same solution as used by Schibsted (2) for the fat aldehyde test—a 0·001% cresol-red solution buffered to *p*H 8·3. The buffer solution is made by dissolving the following salts in distilled water and making to 1 l.:

	g.
Sodium borate decahydrate	7·5477
Boric acid	7·5044
Sodium chloride	1·7696

A stock solution consisting of 0·1% cresol red in buffer solution is prepared; for use this is diluted 1 : 100 with buffer solution.

(5) *Standard ferric iron solution*

A solution containing 1 mg. of iron/ml. is made by dissolving 0·500 g. bright iron wire in iron-free hydrochloric acid and oxidizing with hydrogen peroxide. The excess peroxide is boiled off and the solution diluted to 500 ml. This solution is diluted and acidified to contain 10 mg. of iron and 1·0 ml. 10 *N* hydrochloric acid per 100 ml.

Apparatus

(1) *Glassware*

Glass apparatus is cleaned satisfactorily by brushing with soap and water, thoroughly rinsing with tap water and then distilled water, and by drying in an oven at 100° C.

When apparatus, such as graduated test-tubes and filter funnels, are used repeatedly on the same day, they may be cleaned between tests by rinsing with the benzene-methanol reagent.

(2) *Filter papers*

Extraction of iron from the filter papers is necessary. This may be done as follows. Whatman no. 41, 9 cm. filter papers are folded and stacked in a 2 in. diameter vertical glass tube of about 15 in. length, connected at the bottom to a filter flask and vacuum line. Successive quantities of about 0·5 *N* hydrochloric acid, distilled water and methanol are drawn through the filter papers. The tube and contents are transferred to an oven and dried at 100° C. Care is taken in subsequent storage and handling of the papers to prevent contamination with iron.

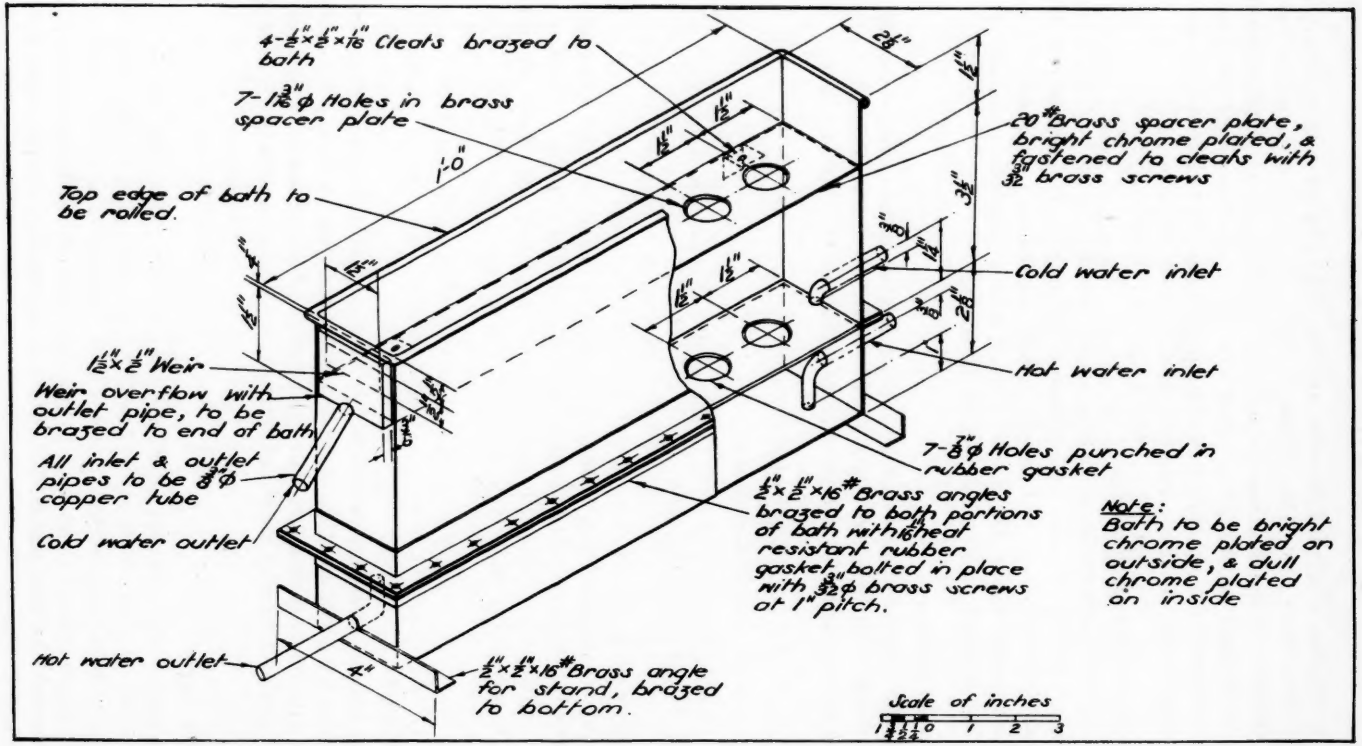
4-1/2"×1/2"×1/16" Cleats brazed to bath
7-13/16" ϕ Holes in brass spacer plate
1'-0"
Top edge of bath to be rolled.
1/2"×2" Weir
Weir overflow with outlet pipe, to be brazed to end of bath
All inlet & outlet pipes to be 3/8" ϕ copper tube
Cold water outlet
Hot water outlet
4"
1"×1"×16# Brass angle for stand, brazed to bottom.
20# Brass spacer plate, bright chrome plated, & fastened to cleats with 3/32" brass screws
Cold water inlet
Hot water inlet
7-7/8" ϕ Holes punched in rubber gasket
2"×2"×16# Brass angles brazed to both portions of bath with 1/16" heat resistant rubber gasket, bolted in place with 3/32" ϕ brass screws at 1" pitch.
Note: Bath to be bright chrome plated on outside, & dull chrome plated on inside
Scale of inches
0 1 2 3

Fig. 1. Test-tube extraction bath.

(3) *Extraction bath.*

For the extraction of the tubes, a bath was constructed as shown in figure 1. This bath was made of brass and was constructed without soldered joints. The bath was provided with a weir at the lower end, which was adjusted to maintain a water level of 2 inches, which

(4) *Constant-temperature water supply.*

A Knight constant-temperature water supply was used. The tube a was connected to the cold-water inlet and tube b to the hot-water inlet were made of 3/8-inch copper tubing. The water was delivered to the bath with which a flow of 500 cc per minute was maintained.

(1) *Cleaning the tubes.*

A weighed tube was filled with 10 cc of 1:1 nitric acid from a pipette and allowed to stand for 5 minutes. The acid was then drained the fatty matter was removed by rinsing with water and drying. The a weighed tube was filled with 10 cc of 1:1 nitric acid from a pipette but did not stand for 5 minutes. The acid was then drained and ± 2% with 10 cc of 1:1 nitric acid from a pipette and allowed to 45° C. for 5 minutes. The acid was then drained and the fatty fine tip was inserted in the tube and the tube was filled with from the tube. The tube was then rinsed with water and dried piece of rubber tubing was placed over the end of the tube out at the end of the tube. The tube was then rinsed with the tube just filled with water and the water drained out. checked ± 2% contained 10 cc of 1:1 nitric acid from a pipette extremities with brass

After solution solve and th with s yellow blanks stands solutio tube a for 2 strengt

(3) *Extraction bath*

For extracting the fat from milk powder, it is convenient to use a bath of the type shown in Fig. 1. By this means the milk powder can be refluxed with solvent in a tube without using a separate condenser. Water over 65° C. or steam can be used to heat the lower part of the tube, while cold water circulates around the upper part of the tube which then acts as a condenser.

(4) *Colorimeter*

A Klett Beaver *p*H colorimeter was used for most of the work here described. The extra tube and plunger supplied with this type is essential for the test. A few measurements were made with a Coleman double monochromator spectrophotometer. These agreed well with values found with the visual colorimeter. Maximal absorption was at a wave-length of 500–510 mμ.

Procedure

(1) *Clear butterfat*

A volume of benzene-methanol equal to 9·9 ml. less the volume of fat to be taken is pipetted into a $\frac{3}{4}$ in. test-tube. The volume of fat taken depends on the peroxide value of the fat. For values between 0·5 and 5·0, 0·1 ml. is convenient. A preliminary dilution of a weighed amount of fat in benzene-methanol may be used in measuring this quantity, but direct measurement of 0·1 ml. of the highly viscous oil can be made with an error of ±2% by the technique described below. For the larger volumes (0·2–1·0 ml.) required with low peroxide values, direct measurement is always used. The butterfat at about 45° C. is drawn into a warmed graduated straight-sided 1 ml. pipette of narrow bore and fine tip, to a little above the lowest relevant mark. The tip of the pipette is wiped free from fat with absorbent cotton and the meniscus adjusted to the mark with the small piece of cotton held against the side of the tip of the pipette, the fat being allowed to flow out at the rate of 0·01 ml./sec. The same rate of outflow is used in delivering the fat into the test-tube containing solvent, the tip of the pipette being held against the side of the tube just above the benzene-methanol. The quantity of fat delivered by this method is checked by weighing several such lots pipetted into a weighing bottle. An accuracy of ±2% is obtained if the same pipette is used for all samples. This introduces no measurable contamination if the pipette is well drained and then rinsed with the next fat. Where extreme variation is to be expected between successive samples the pipette may be rinsed with benzene-methanol.

After measuring the fat into the solvent, 1 drop (0·05 ml.) of ammonium thiocyanate solution and 1 drop of ferrous chloride solution are added. The test-tube is shaken to dissolve the fat and water, placed for 2 min. in a water-bath at 50° C. to develop the colour and then cooled to 25° C. In the Klett *p*H colorimeter the tube below the standard is filled with suitably diluted potassium chromate solution, and its depth adjusted to match the yellow colour of the butterfat. This compensating tube is also required for matching the blanks. The artificial standard should be checked for every series of tests against an iron standard. This is prepared by diluting 0·5 ml. of the 0·1 mg./ml. aqueous standard iron solution to 50 ml. with benzene-methanol: 10 ml. of this solution are pipetted into a test-tube and 1 drop of ammonium thiocyanate is added. The solution is shaken, held at 50° C. for 2 min., then cooled and compared in the colorimeter with the artificial standard. The strength of the artificial standard is calculated in terms of μg. of iron/10 ml.

A blank without fat but otherwise subjected to the same treatment as the unknowns is required for each batch of tests. There is also some red component in the butterfat colour, and this, together with any iron in the fat, must be allowed for by using an additional blank containing fat and thiocyanate, but no ferrous salt. Because there is appreciable variation in this quantity, it is necessary to determine separately the fat blank value for each fat when the peroxide value is low. The value corresponding to 0·1 ml. of fat is usually equivalent to about 0·25 μg. of iron/10 ml.

Fat and reagents are protected from light during the test. Use of a semi-darkened room has given satisfactory results. Precautions are also taken against iron contamination. Delay at any stage is avoided. About twenty unknown solutions may conveniently be put through in a batch.

The peroxide value (P.V.) is expressed as m.equiv. of oxygen per kg. of fat. The concentrations of the unknown and blanks are calculated in terms of μg. of iron/10 ml., and the net figure for the unknown is then obtained by deducting the blanks:

$$\text{P.V.} = \frac{\text{Net } \mu\text{g. iron/10 ml.}}{\text{G. of fat taken} \times 55{\cdot}84}.$$

(2) *Butter*

A clear butterfat may often be obtained from butter by placing a quantity in a 30 ml. centrifuge tube, melting slowly by immersion in a water-bath at 40° C., and then centrifuging. When this procedure fails to give a clear fat it may be followed by filtration through a treated filter paper in an oven at 45° C. The time the fat is held melted before testing, and exposure to light, should be reduced to a minimum. The remainder of the test is carried out as under section (1) above.

(3) *Milk*

A pint of milk is centrifuged in two 250 ml. centrifuge bottles to give a firm cream layer. The skim milk is pipetted from below the cream, both lots of cream are combined in one bottle with enough skim milk to make the cream fluid, and the cream is then churned by shaking the bottle in a shaking machine. After the buttermilk has been drained off, the butter granules are washed and then transferred to a 30 ml. centrifuge tube and the method for butter then followed.

(4) *Milk powder*

Fifteen ml. of benzene-methanol are pipetted into an 8 × 1 in. test-tube, and 0·75 g. of milk powder are weighed out on an aluminium weighing dish (e.g. a milk bottle top), shaped to allow the powder to be poured into the tube. The powder is dispersed through the solvent by shaking, and to prevent caking this is repeated at intervals until all the tubes in the batch are placed in the extraction bath. Included in each batch in the extraction bath is one tube containing solvent, but no milk powder. Cold water is circulated through the upper section of the bath, and then hot water through the lower section. Extraction is continued for 5 min. from the time the middle tube commences to boil. Cold water is then circulated for 2 min. through the lower section.

The solution of fat in benzene-methanol is filtered through a treated filter paper into a $\frac{1}{2}$ in. internal diameter test-tube marked at 10 ml. When 10 ml. of filtrate have been obtained, the filter funnel is removed. One drop of ammonium thiocyanate and 1 drop of ferrous chloride are then added to the fat solution. The tube is stoppered with a rubber

stopper (chances of contamination are reduced if one stopper is reserved for this purpose and well rinsed with solvent before and after use) and shaken to dissolve the water. The tubes are then immersed in a water-bath at 50° C., and the determination then completed without delay by comparison in the colorimeter as described for clear butterfat. Where peroxide values are very low, fat blanks should be determined by carrying out extraction in the normal manner, but adding no ferrous chloride. The usual value for a fat blank will be 0·3 μg. of iron/10 ml. of solvent. Peroxide value is then calculated as m.equiv. of oxygen per kg. of milk powder:

$$\text{P.V.} = \frac{\text{Net } \mu\text{g. of iron/10 ml.}}{(\text{Wt. of powder} \times \frac{10}{15}) \times 55{\cdot}84}.$$

Development of method

Solvent

The 96 % acetone used as solvent for the ferric thiocyanate test by Lips *et al.* (4) was found to have three disabilities when applied to the study of low peroxide values ($< 0{\cdot}5$): the solubility of fat is low (about 0·05 g./10 ml.), the reagent when ready for use shows some colour, and the reagent is unstable—blank and test values increasing markedly during the necessary heating and also on standing. It was thought that these disabilities might be overcome by using as solvent a mixture of a good fat solvent and an alcohol, which as well as permitting the use of a greater concentration of fat might reduce the blank value by allowing addition of the inorganic reagents in aqueous solution at the time of carrying out the test. Several such solvents were tried, and a mixture of 70 vol. of chloroform and 30 vol. of ethanol was found to be quite suitable for the purpose. This solvent was used for much of the work on low peroxide values in fresh milk and cream, including most of those reported in Fig. 2. Blank values were usually about 0·2 μg. of iron/10 ml. When the solvent was used for milk powder it was found that an extraction period of 50–60 min. was necessary to obtain maximal values, as against 20 min. with acetone. A solvent consisting of acetone and chloroform (9 : 1) was also tested. It showed good fat solubility but even less stability than acetone alone.

Later the solvent of Bolland *et al.* (6) (73 % benzene, 27 % methanol by volume) which had been developed for study of peroxide in rubber was also found to permit the addition of the inorganic reagents in aqueous solution. The solubility of water in this solvent was about 2·5 %, the same as in chloroform-ethanol. Use of this solvent instead of chloroform-ethanol was adopted for several reasons, in addition to its prior use for a similar purpose. The price is much lower, the colour of the ferric thiocyanate solution is less yellow, permitting better comparison in the presence of butterfat in the visual colorimeter (both solvents are superior to 96 % acetone in this respect), and the extraction period for milk powder is only 5 min. The proportion of methanol was increased slightly to 30 % to give a greater water solubility.

Optimal hydrogen-ion concentration

With all solvents tested, maximal colour intensity of the ferric thiocyanate was found to be dependent on the hydrogen-ion concentration. This was particularly noticeable with acetone, and at one stage obvious variation in the colour of the iron standards was traced to absorption of ammonia from the air of the laboratory. Even acetone freshly distilled from calcium chloride did not always give maximal colour with ferric iron. Acetone

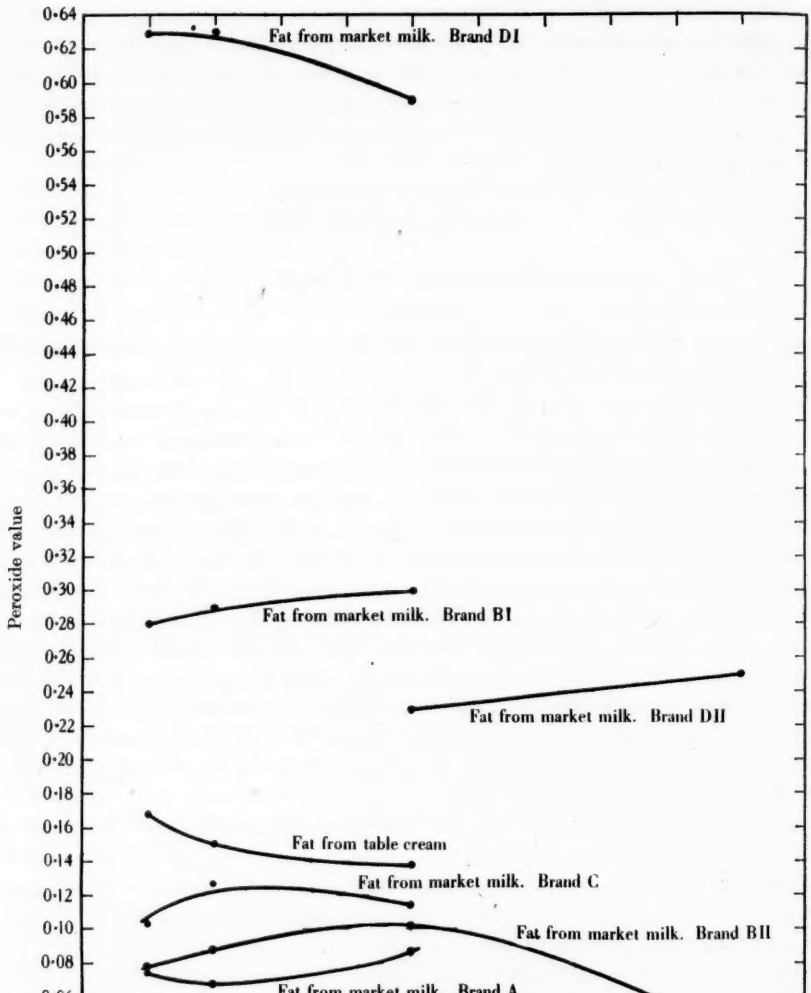

Fig. 2. Peroxide values in fresh market milk and cream showing variations with concentration of fat used in the test.

acidified with hydrochloric acid to give a final solution reading *p*H 3·1 with the glass electrode was satisfactory. Typical figures for variation of colour intensity with addition of hydrochloric acid are given in Table 1. These figures are for chloroform-ethanol. A concentration of hydrochloric acid of 0·0035% was found to give maximal colour with benzene-methanol. At first the practice of adding the acid to the solvent was adopted. Heating fat with this acidified solvent was found to destroy peroxide, while heating with neutral solvent caused no destruction. This is shown by the results listed in Table 2.

Table 1. *Effect of hydrogen-ion concentration on colour intensity*

% HCl added	Glass electrode reading of final solution	Colour intensity as g. Fe/10 ml.
Nil	4·2	11·6
0·000018	4·1	14·3
0·00018	3·95	15·3
0·0018	3·26	15·4
0·018	2·30	15·4
0·036	1·95	15·7
0·20	—	Very low

Table 2. *Effect on the peroxide value of refluxing with neutral and with acidified solvent*

Time of refluxing (min.)	Peroxide value: Benzene-methanol containing 0·0035% HCl	Peroxide value: Neutral benzene-methanol
Zero	1·83	2·07
Zero	1·84	1·97
10	0·86	2·07
50	0·36	1·94

Neutral solvent was therefore used, and the required acid concentration in the final test solution was obtained by suitably acidifying the ferrous chloride. It may be noted that whereas 0·0035% HCl in benzene-methanol gives a *p*H value of zero with the glass electrode, the same solution containing 1 drop of 30% ammonium thiocyanate will read about *p*H 3·0. Thus, if the thiocyanate is added at the same time as the acidified ferrous chloride, destruction of peroxide is not to be expected.

Addition of ferrous salt

Solutions of ferrous salts in organic solvents oxidize much more rapidly than in acidified aqueous solution. To reduce blank values and improve stability, it was therefore desired to add the ferrous salt in aqueous solution at the time of carrying out the test. The addition of 1 drop of 0·5% aqueous ferrous ammonium sulphate to 10 ml. of 96% acetone containing 0·4% ammonium thiocyanate caused cloudiness, suggesting incidentally that the solubility of ferrous ammonium sulphate in this solution is far below the 0·1% which is added in the method of Lips, Chapman & McFarlane. The addition of 1 drop of 0·5% ferrous sulphate acidified with sulphuric acid to chloroform-ethanol resulted in a slight cloudiness. When the solution was acidified with hydrochloric acid the cloudiness was reduced. This led to the use of ferrous chloride in place of ferrous sulphate, and it proved possible to add ferrous chloride solution to all solvents used without precipitation. Preparation of ferrous chloride by solution of iron in hydrochloric acid gave an excessive

content of ferric iron. The method of interacting barium chloride and ferrous sulphate was then adopted with very satisfactory results.

Concentrations of ferrous iron between 10 and 460 μg./10 ml. were found to affect the determination of hydrogen peroxide, but had little effect on the determinations of peroxides in fat. Relevant figures are given in Table 3. The more concentrated ferrous solutions gave higher blank values, and for this reason use of the dilute solution was continued. For very low peroxide values ($< 0{\cdot}1$), a more dilute solution (0·05% as $FeCl_2$) may be used.

Thiocyanate concentration and order of addition

The thiocyanate concentration in the test solution is 0·15% compared with 0·18% used by Bolland *et al.* (6) for peroxide determinations in rubber and 0·4% used by Lips *et al.* (4) for peroxides in fats. Reducing the concentration to 0·07% noticeably reduced the colour intensity, but an increase to 0·30 or even 0·45% did not increase the colour intensity.

Table 3. *Variation of peroxide value with concentration of ferrous iron*

Conc. ferrous solution as % $FeCl_2$	μg. iron in 10 ml. final test solution	Net μg. of ferric iron					
		Blank	*Hydrogen peroxide	Fat A peroxide	Fat B peroxide	Fat C peroxide	Fat D peroxide
0·05	10	0·2	—	—	—	0·23	0·024
0·23	46	0·4	8·6	17·2	5·28	0·22	0·027
1·15	230	0·8	10·3	17·8	—	—	—
2·3	460	1·7	11·0	18·1	5·56	—	—

* The amount of hydrogen peroxide added was theoretically equivalent to 11·7 μg. of iron.

Table 4. *Effect of variation in fat concentration on the estimated peroxide value*

Volume of fat per 10 ml.	Peroxide value			
	Fat A	Fat B	Fat C	Fat D
0·1 ml.	0·24	0·72	0·12	0·17
0·2 ml.	0·30	0·68	0·10	0·22
0·5 ml.	0·38	0·69	0·08	0·18

If the ferrous salt is added to the solution of fat in benzene-methanol and the mixture is then warmed before adding the thiocyanate, high values are obtained. Oxidation by the fat peroxide appears to accelerate autoxidation of the ferrous salt in the absence of thiocyanate. This is particularly apparent when hydrogen peroxide is used in place of fat peroxide. The normal method of adding both ferrous chloride and ammonium thiocyanate before shaking to dissolve the aqueous solutions excluded error from this source.

Consistency of results and effect of fat concentration

When duplicate tests are carried out using the same concentration of fat, agreement within ±3% may be expected. Variation in fat concentration up to 0·1 ml./10 ml. of benzene-methanol has little effect on the peroxide value obtained. For peroxide values below 0·2, however, it is necessary to use higher fat concentrations to give sufficient colour intensity. With volumes of fat from 0·1 to 1·0 ml./10 ml. of solvent considerable variation in the estimated peroxide value of the fat is observed. Typical variations are shown in Fig. 2. Some of these were with chloroform-ethanol and some with benzene-methanol. Another series of figures for chloroform-ethanol is given in Table 4. The variations are not always in the same direction and, indeed, the same fat on holding for a day may change in direction of variation.

The cause of the variations has not been clarified. That they were not due to differences in the peroxide concentration was indicated by lack of regularity in the variations. Further evidence on this point was obtained by using different volumes of a more highly oxidized fat diluted 1 : 80. The figures obtained are given in Table 5. The possibility that the alteration of peroxide value with different fat concentration was an optical effect, due to the fat colour, was then investigated. A standard ferric thiocyanate solution was tested in the presence of 0·1–0·5 ml. of butterfat/10 ml. After allowing for the red component in the fat colour, there was no difference in the red colour intensity. When different concentrations of fat of low peroxide value were tested in the presence of a constant small amount of highly oxidized fat, the variation in the total peroxide determined was similar to the variation when the low peroxide fat was tested alone. Whatever is responsible for variation of peroxide value with a particular fat does not appear to influence the reaction of any other fat peroxide present.

The addition of copper in concentrations likely to be encountered in butterfat did not affect the peroxide value in any way. Use of higher ferrous chloride concentrations did

Table 5. *Effect of variation in peroxide concentration on the peroxide value*

Volume of fat solution (ml.)	μg. of iron/10 ml.		
	Gross	Net	Peroxide value
0·1	2·14	1·97	1·99
0·2	3·90	3·73	1·89
0·5	8·96	8·79	1·80
1·0	18·17	18·00	1·82
Blank	0·17	—	—

Table 6. *Time of extraction of peroxide from whole milk powder with neutral benzene-methanol*

Extraction times (min.)	Peroxide value			
	Spray powder	Spray powder	Spray powder	Roller powder
0	0·31	—	—	—
2	0·92	0·06	0·09	0·27
5	0·96	0·11	0·14	0·25
10	0·93	0·08	0·14	0·25
30	0·67	0·03	0·11	0·22

not alter the variations. Traces of buttermilk solids sufficient to cause a slight cloudiness in the fat were found to cause marked falling off in peroxide value with fat concentration in one case, but even when the fat was filtered and became perfectly clear, appreciable variation persisted.

Because of these differences in peroxide value with fat concentration it has been customary when studying fat of low peroxide content to use two or three concentrations of the fat.

Modification of the test for milk powder

On extracting the fat from milk powder with benzene-methanol, maximal peroxide values were obtained after 5 min. After 2 min. extraction was not complete, and after 10 min. there was some decrease in the value obtained. It has already been noted that with clear fat, destruction of peroxide occurred in acidified solvent but not in neutral solvent. With milk powder, however, destruction occurred even with neutral solvent, but the decrease in peroxide value for 10 min. extraction compared with 5 min. is small (see Table 6). Also it was found that 96% of the butterfat in spray-dried milk powder was

extracted in the first 5 min. and practically no more during the second 5 min., so that the observed decreases in peroxide value between 5 and 10 min. cannot be due to a large destruction of peroxide accompanied by extraction of a compensating amount of fat. The rate of destruction of peroxide is therefore low, and the amount destroyed in the first 5 min. is probably negligible. Where reliable results using acetone as a solvent were obtained, the peroxide values agreed closely with those obtained with benzene-methanol.

In an attempt to confirm the validity of the extraction method, peroxide values estimated on fat pressed out from milk powder were compared with values obtained by solvent extraction. The spray-dried milk powder was pressed in a 2 in. diameter die placed on two prepared filter papers resting on a stainless steel plate. At a pressure of 6–8 tons/sq.in., sufficient fat was squeezed out of the milk powder to saturate the two filter papers, the lower one of which could be stripped off readily. The fat was dissolved out of this paper with benzene-methanol and the peroxide value estimated in the usual way. In a few cases the peroxide values obtained by the two methods agreed well, but usually the values obtained with the expressed fat were 20–100% greater than those obtained by solvent extraction. It was thought that the discrepancies might be due to the liquid fractions of the butterfat containing higher proportions than normal of oleic acid being expressed, but this was disproved by estimations of iodine values. As only a small proportion of the total fat can be pressed out by application of direct pressure it is possible that this fat is loosely held on the outsides of the powder particles and therefore more likely to oxidize than the fat enclosed in the particles. The only other likely explanation of the discrepancy is that peroxide is extensively destroyed during extraction with solvent, but the evidence given previously that such destruction does not occur indicates with reasonable certainty that the extraction procedure gives a true measure of the fat peroxide.

In earlier work when chloroform-alcohol was being used as solvent the filtrate from milk powder sometimes clouded, either before or after the inorganic reagents were added. Provided the solvent contained at least 0·5% water, cloudiness did not occur, and although clouding has not been observed with benzene-methanol it is possible that it might occur with certain milk powders. Addition of water after the cloud had formed had no effect, which led us to believe that it was correlated with the physical state of the lactose in the milk powder. It is known that solutions of lactose in alcohol supersaturate readily and crystallize slowly.

It has been mentioned previously that the estimated peroxide value varies with some butterfats if more than 0·09 g. of butterfat/10 ml. of solvent are taken. The same effect is noticed with milk powder, so that the amount of powder used for the test should be stated. Unless the peroxide value is very high 0·75 g. of powder extracted with 15 ml. of solvent and 10 ml. of filtrate taken is satisfactory. This corresponds to about 0·125 g. of butterfat in the final 10 ml. of solution.

The liquid obtained from the milk powder after extraction must be quite free from suspended material or the ferric thiocyanate colour does not develop fully in the final stages of the test. With care a sufficiently clear solution may be obtained by decantation, but there is an element of uncertainty that is avoided if filtering through paper is adopted. Unfortunately, filter papers as bought contain varying amounts of iron which must be removed if consistent results are to be obtained. The top paper and one other from eight boxes of Whatman no. 41 filter papers were washed with 10 ml. of cold solvent, and it was found that 0·10–0·57 μg. of ferric iron were extracted. Two further papers from each box were washed with dilute hydrochloric acid, distilled water and methyl alcohol, after

which treatment no iron was extracted by the solvent. Details are given under the section on apparatus and reagents for preparing the papers in an iron-free state in quantity.

Table 7. *Peroxide values of milk powder. Reproducibility*

Sample	1st series	2nd series	3rd series
E	0·77	0·77	0·81
	0·77	0·81	0·81
D	0·23	0·25	0·25
	0·24	0·24	0·25
A	0·40	0·40	0·40
	0·42	0·42	0·41
HN	0·019	0·022	—
	0·020	0·022	—
	0·020	0·021	—
ST	0·018	0·020	—
	0·015	0·023	—
	0·017	0·022	—

As applied to milk powder the test is reasonably reproducible. Duplicate determinations were carried out on five milk powders and then this series of determinations was repeated. The values obtained are recorded under first and second series in Table 7. The two powders HN and ST gave low peroxide values and the results have been recorded to the third decimal place in order that the extent of the variation may be seen. In a third series of replications the samples were left standing 15 min. after extracting and cooling but before filtering, and then a further 15 min. just before reading but after the final colour had been developed. The values obtained were not altered by this rather drastic treatment.

Comparison of iodometric and thiocyanate test

Determination of fat peroxide values by both the method described and the Lea iodine method (1) on over 100 different fats has shown a ratio between the two tests varying from 1·5 to 2·5 and averaging a little over 2·0. This agrees with the ratio found by Lips *et al.* (4) for ferric thiocyanate to iodometric peroxide values. This ratio has held even for slightly oxidized fats (P.V. $< 0{\cdot}5$), where the concentrations of oxidation products other than the peroxide are almost certainly low.

Lips *et al.* (4) suggest that heating in glacial acetic acid and chloroform, as for the Lea test, causes destruction of peroxide, and that this explains the lower values. Fat was heated with glacial acetic acid and chloroform as for the Lea test, but without potassium iodide, and the acetic acid then washed out. An aliquot of the remaining fat in chloroform was taken for a thiocyanate peroxide test. The values obtained gave no evidence of appreciable destruction of peroxide.

	Before boiling with acetic acid-chloroform	After boiling with acetic acid-chloroform
Fat A	0·20	0·19
	0·20	0·21
Fat B	5·4	5·3

Fat which had been interacted with hydriodic acid in the Lea test was washed free from acetic acid and liberated iodine. Peroxide values on the remaining solution of fat in chloroform made by both iodine and thiocyanate procedures were as follows:

	Initial peroxide value		Peroxide value after Lea test	
	Lea	Thiocyanate	Lea	Thiocyanate
Fat A	2·30	—	0·14	0·24
Fat B	2·88	5·4	0·18	0·36

This very incomplete work on the relation of the two tests suggests that the substances responsible for the thiocyanate peroxide values are altered by the hydriodic acid in the Lea peroxide test, but without releasing a corresponding amount of iodine.

Application of the test

Milk

On two occasions samples were milked from a cow into a glass container, and protected from light during transit to the laboratory. The fat was separated by the method previously described and immediately tested for peroxide. The total time elapsing from milking to completion of the test was about 2½ hr. Both samples gave peroxide values a little above 0·01. These values and the higher figures obtained for the same fat held in semi-darkness at room temperature for a further 3 hr. are shown in Fig. 2, which also shows values ranging from 0·06 to 0·30 obtained for various samples of fresh market milk. The values obtained demonstrate that the test is sufficiently sensitive to permit oxidation of the fat to be followed from the time the milk leaves the udder. It is possible that this peroxide test may provide a quantitative measure which has hitherto been lacking in studies of oxidized flavour in milk, even if oxidation of phospholipid is responsible for the flavour change.

Butter

Several samples of fresh butter have been examined. Peroxide values ranged from 0·02 in a choicest butter 5 days old to 0·17 in a first-grade butter held 14 days at room temperature. Study of the peroxide value of butters is to be continued to determine whether correlation exists with oily, fatty and flat flavours which are usually regarded as the first suggestion of oxidation.

Butterfat and oxidation studies

Samples of butterfat at various stages of oxidation were examined. It was observed that with fat held in air at room temperature the first flavour change can usually be detected when the peroxide value is between 0·3 and 0·8. This degree of oxidation normally causes a flattening or loss of fresh flavour rather than the appearance of a definite off-flavour.

A sample of fresh butterfat was allowed to oxidize, and thiocyanate and Lea peroxide values were determined at intervals. The results which afford a comparison of the sensitivity of the two methods appear in Table 8.

Table 8. *Relative sensitivity of thiocyanate and Lea peroxide tests*

Fat	Peroxide value	
	Thiocyanate	Lea
Original fat	0·028	No colour
After 4 min. exposure to dull daylight	0·067	No colour
After further 8 min. exposure to dull daylight	0·14	0·045
After further 24 hr. in room light	0·25	0·10

The greater sensitivity of the ferric thiocyanate test has been made use of in this laboratory in studies of the oxidation of butterfat at 40° C. It has been possible to obtain reliable indications of relative oxidation after 3–4 days' incubation instead of the 7–12 days previously required. In addition to the saving of time, the low degree of oxidation calls

for less oxygen than is dissolved in the fat, and the uniform and adequate supply of oxygen thus ensured should give greater accuracy.

Milk powder

No systematic study of the peroxide values of milk powders stored under various conditions has been made, but at a peroxide level of 0·6 oxidized flavours were noted in samples stored in friction lid tins for 3 months at 30° C. Chapman & McFarlane(3), working with two samples of milk powder also stored in friction lid tins, but held at room temperature, did not detect oxidized flavours until the peroxide value had reached 5. This would correspond to a peroxide value in the fat of 20, or roughly 10 by the Lea test, and in our experience butterfat which has oxidized to this extent is extremely tallowy and usually bleached. It is likely that flavour change would occur, even with the masking effect of the flavour of other milk solids before this degree of oxidation was reached.

SUMMARY

The ferric thiocyanate method of estimating peroxide in fats has been modified to give greater sensitivity and greater stability of the reagent. As the 96% acetone used by Chapman & McFarlane(3) and Lips *et al.*(4) only dissolves about 0·5% butterfat, the sensitivity of the test was increased by using, as solvent, the mixture of benzene and methanol used by Bolland *et al.*(6) in the determination of rubber peroxides, which dissolves at least 10% butterfat. Instead of using a prepared reagent which gradually deteriorates, the ferrous salt and thiocyanate are added in aqueous solution during the test, and, moreover, ferrous salts appear to be more stable in this solvent than in acetone. The modified method is sufficiently sensitive to measure the peroxide value of the fat of freshly drawn milk and has been applied to the study of the first stages of oxidation in milk, butter and whole-milk powder.

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339. THE CONSUMPTION OF MILK AMONG FOUR SOCIAL CLASSES OF CONSUMERS

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PART I. THE CONSUMPTION OF LIQUID, TINNED AND DRIED MILK

In surveys of milk consumption in which the relevant information is collected from a sample of housewives, two methods have, broadly, been used for selecting the families to be studied. These can best be described by actual examples. On the one hand, Murray (1) selected a random sample of families in the city of Oxford, and in 1938–9 Murray & Rutherford (2) made a similar selection of families in seventeen towns in different parts of England. On the other hand, Bransby (3) studied four different social classes of consumers in Sussex and Yorkshire, and in 1935–6 Bransby (4) studied 300 slum families. Other workers (Jones & Cowie (5), Harry (6), Makings (7)) have also selected samples according to social class. That different results are obtained by the two methods of selection is demonstrated by comparison of the statistical analyses of Murray (1) and Bransby (4). The present study was made to examine the milk-consumption characteristics of four social classes of consumers in the city of Leeds.

Collection of data

Families of the following four social classes were surveyed:

Lower working class. All the houses were condemned as unfit for human habitation. Householders followed unskilled occupations.

Working class. The houses were not condemned. The householders followed more skilled occupations than did those of the lower working class.

Lower professional class. Typical householders were clerks, insurance agents and commercial travellers.

Professional class. Householders held higher grade appointments such as University staff, barristers and company directors.

Data were collected during interviews with housewives by well-trained and experienced field workers. Information was obtained from 150, 152, 124 and 135 lower working, working, lower professional and professional class families respectively. The percentage of refusals to provide complete information, including that on housekeeping expenditure, was 15, 18, 27 and 26 % for the four groups. The field work was done during May–June 1939. Housekeeping expenditure was the money the housewife had for housekeeping, and not total family income or expenditure.

Results

Table 1 gives data relating to family size, housekeeping expenditure and food expenditure, one somewhat surprising feature of the table being the small differences of family size in the four groups. Table 2 shows consumption of the various kinds of milk per head weekly for the four social classes during the investigation. It will be noticed that total milk consumption per head weekly increased from the lower working to the professional class. The importance of tinned milk in raising average consumption among lower working

class families is well demonstrated. The percentages of household expenditure spent on milk are given in Table 3, showing that apart from a small decline among lower professional class families the percentages of food expenditure spent on milk were fairly constant at about 9·5 % in all classes.

Table 1. *Family composition and expenditure according to social class*

Social class	No. of families	Adults	No. of children: Up to 5 yr.	No. of children: 5–14 yr.	Persons per family	Housekeeping expenditure: Per head s.	Housekeeping expenditure: Per head d.	Housekeeping expenditure: Per family s.	Housekeeping expenditure: Per family d.	Food expenditure: Per head s.	Food expenditure: Per head d.	Food expenditure: Per family s.	Food expenditure: Per family d.
Lower working	150	330	58	70	3·05	12	8	39	5	6	9	20	6
Working	152	417	28	66	3·36	15	11	53	6	8	2	27	7
Lower professional	124	359	20	19	3·21	—		—		12	4	39	5
Professional	135	417	23	51	3·64	—		—		14	4	52	1

Table 2. *Consumption of milk (in pints) according to social class*

Social class ...	Lower working: Per head per week	Lower working: Cost £ s. d.	Working: Per head per week	Working: Cost £ s. d.
Liquid milk in household	1·58	8 14 3	2·61	16 16 4½
Liquid milk in school	0·26	10 5	0·17	11 0½
Tinned milk	0·91	4 8 9½	0·42	2 10 9
Dried milk	0·12	16 6	0·02	4 0
Skimmed and goats' milk	0·01	1 8	0·02	1 10
Total	2·88	£14 11 7½	3·24	£20 4 0

Social class ...	Lower professional: Per head per week	Lower professional: Cost £ s. d.	Professional: Per head per week	Professional: Cost £ s. d.
Liquid milk in household	4·32	20 18 6½	4·95	31 5 5
Liquid milk in school	0·10	5 0	0·14	8 5½
Tinned milk	0·07	9 8	0·14	1 0 9
Dried milk	—	—	0·03	7 8
Skimmed and goats' milk	—	—	—	—
Total	4·49	£21 13 2½	5·26	£33 2 3½

Table 3. *Percentage of household income and food expenditure spent on milk*

Social class	Expenditure (%): Housekeeping	Expenditure (%): Food
Lower working	4·9	9·4
Working	5·0	9·6
Lower professional	—	8·8
Professional	—	9·4

THE CONSUMPTION OF LIQUID MILK

(*a*) *In school.* Of the 242 children attending school where school milk was available, 164 purchased milk and twenty-two received it free of charge. About 80 % of the lower working, working and lower professional class children and 60 % of the professional class children took milk. The main reason given for non-participation in the milk in schools scheme was cost among lower working class families, and such reasons as milk spoiled dinner or was not of sufficiently good quality in the other classes.

To determine the influence of housekeeping income on the consumption of milk at school, the children attending school of each social class were divided into two groups, according to the food expenditure of the families to which they belonged. These data for

each social class were analysed statistically (by the χ^2 test), and no significant difference was found for any class between the proportion of children above and below the defined expenditure limits who took milk at school.

A further analysis was made by determining regression equations relating the number of children consuming milk in school with the number of adults and children in the family, the number of children attending the school and the food expenditure of the family. The only significant coefficients were those of 'the number attending school' and they were of the order of 0·8, the differences between the social classes not being significant. The multiple correlation coefficients between the consumption of milk in school and the factors mentioned above were 0·71, 0·80, 0·56 and 0·73 for the lower working, working, lower professional and professional classes respectively, which indicates that no other factor had any appreciable influence on the consumption of school milk.

(*b*) *At home.* Before detailing the results of the analysis of the factors influencing the consumption of liquid milk, it is important to emphasize that among lower working class families, tinned milk was the basis of the household milk supply, and that liquid milk was subsidiary. Among the other social classes, liquid milk was the basic supply.

Factors influencing consumption

Regression equations were determined for the social groups relating liquid milk consumption with the number of adults and children in the family and with family food expenditure and housekeeping expenditure. The results are given in Table 4.

Table 4. *Regression coefficients relating liquid milk consumption to family constitution and household income and food expenditure*

Social class ...	Lower working	Working	Lower professional	Professional
Adults	−0·14	1·26*	2·19*	1·85*
Children	0·35	0·48	4·28*	3·75*
Housekeeping expenditure	0·10*	0·07*	—	—
Food expenditure	0·16*	0·09*	0·13*	0·22*

* Represents that the coefficient is significant.

Family constitution. Table 4 shows that consumption was independent of family constitution among lower working class families. Among those of the other social groups it increased by about $1\frac{3}{4}$ pt. per week for every additional adult in the family. Among working class families consumption was independent of the number of children in the family, but among the two professional classes it increased by about 4 pt. per week for every additional child. Lower working and working class families did not increase the family milk supply according to the nutritional needs of the children in the family, whereas the lower professional and professional class families did so.

Food expenditure and housekeeping expenditure. Table 4 shows that milk consumption increased among lower working and working class families 1 pt. per week for about every 13*s.* increase in housekeeping expenditure. A 10 % change in expenditure brought a 6·4 and 4·6 % change in consumption among these classes respectively. Consumption increased 1 pt. per week for every 8*s.* increase in food expenditure among the lower working, working and lower professional classes and for every additional 4*s.* 7*d.* among the professional class.

The multiple correlation coefficients between milk consumption and the number of adults and children in the family and food expenditure showed that these three factors

accounted for 18 % of the variations in consumption among the lower working classes, 35 % among the working class and 58–59 % among the professional classes. Therefore at least 41 % of the variations in milk consumption, rising to 82 % in the lower working class families, were due to the 'personal factor' of the housewife. These 'personal factors' are discussed below.

Housewife attitude and knowledge. Milk occupied a much more prominent place in the minds of the housewives of the higher than of the lower social classes, the attitude towards it becoming increasingly active from the lower working to the professional classes. This increase in positive opinion found concrete expression in greater consumption among the higher social classes. Further, the latter had more knowledge concerning the value of milk.

Housewife mentality and consuming habits. In general, there was improvement in the standard of education from the lower to the higher social groups, and this was accompanied by a greater knowledge of cookery and ability to use milk. In slum households the incentive to learn and improve has largely been destroyed by the continual struggle against hardship, but in the other classes there is often the desire for change and the willingness to learn.

Household storage facilities. Only the professional class families had proper facilities for the storage of milk. Among lower working class families they were non-existent and among working class families very unsatisfactory. Waste due to souring tends to depress consumption and to influence housewives to use tinned in preference to liquid milk. It also creates a mental attitude unfavourable to increasing liquid milk consumption.

The price of milk. No data became available in this study on the effect of the price of milk on consumption.

THE CONSUMPTION OF TINNED MILK

Table 5 gives details of the amounts and types of tinned milk consumed. The 'milk equivalent' of tinned milk was taken as that stated on the tins. Tinned milk formed the basis of the household milk supply among lower working class families, and was used for special purposes, such as for puddings, among the professional classes. The real competition between liquid and tinned milk took place among working class families.

Table 5. *Consumption of tinned milk*

Social class ...	Lower working	Working	Lower professional	Professional
	Pints 'milk equivalent' per head per week			
Unskimmed	0·29	0·19	0·06	0·14
Skimmed	0·62	0·23	0·01	—
Total	0·91	0·42	0·07	0·14
	Percentage of total tinned milk consumption			
Unskimmed	32	46	88	100
Skimmed	68	54	12	—
% families using tinned milk	79	53	11	23

Factors influencing consumption

Because of the low consumption of tinned milk among the professional classes, statistical analyses of the data were only made for the working classes. For them regression equations were determined relating the consumption of tinned milk with the number of adults and children in the family and with family food expenditure. The coefficients are given in Table 6.

Table 6. *Regression coefficients relating tinned milk consumption with family constitution and food expenditure*

Social class ...	Lower working	Working
Adults	0·63*	0·27
Children	0·81*	0·24
Food expenditure	0·02	0·01

* Represents that the coefficient is significant.

Family constitution. Table 6 shows that among lower working class families, consumption increased by about ¾ pt. 'milk equivalent' per week for every additional person in the family, but that among working class families consumption was not dependent on family constitution.

Food expenditure. In both the lower working and working classes, consumption was independent of family food expenditure.

The multiple correlation coefficient between tinned milk consumed and the family constitution and food expenditure showed that only 23 and 4 % of the variations in consumption among lower working and working classes respectively were due to these factors. Hence the consumption of tinned milk was due largely to the whim of the housewives.

Table 7 shows the reasons stated by housewives for using tinned milk.

Table 7. *Reasons for using tinned milk*

Social class ...	Lower working	Working	Lower professional	Professional
Convenience	23	23	7	6
Price	49	16	0	1
Preference, cooking and sweets	49	52	27	14

The price of tinned milk and keeping property. The average price per pint 'milk equivalent' was 2·6*d.*, 2·9*d.*, 4·1*d.* and 3·6*d.* among lower working, working, lower professional and professional classes respectively. The prices among the two former were lower than that of liquid milk at 3*d.* per pint, although no data are available to compare the economy of use of the two kinds of milk. The main reason given for using tinned milk by lower working class households was that it was cheaper than liquid milk, but that factor was unimportant in the other groups. Housewives take account of the relative keeping properties of tinned and liquid milk when making this assessment.

Preference and use for particular purposes. There was considerable preference for the flavour of tinned milk among the working classes. Among the professional classes this preference took the form of using tinned milk for cooking or special purposes.

Convenience. Among many lower working and working class families tinned milk was considered more convenient, particularly if some member of the family left for work before the morning delivery of milk. Liquid milk kept overnight might sour.

The consumption of dried milk

The consumption of dried milk was small, 57½, 11½ and 13½ pt. 'milk equivalent', among the lower working, working and professional classes respectively. The price was 3·4*d.*, 4·2*d.* and 7·1*d.* per pint 'milk equivalent' in the three above-mentioned classes. Most of the dried milk consumed by the working classes was obtained from the Child Welfare Centres at a reduced price.

THE TOTAL CONSUMPTION OF MILK ACCORDING TO AGE

A statistical analysis of the data of liquid, tinned and dried milk consumption was made to estimate, for each of the four social classes, the average consumption by children under 5 years of age, children aged 5–14 years and persons over 14 years. Milk drunk at school was included in this analysis. The results are shown in Table 8. There was a significant increase in liquid milk consumption by adults from 1·6 pt. in the lower working to 4·6 pt. in the professional class. Consumption by children under 5 years increased from 1·6 pt. in the lower working to about 4 pt. in the other classes. That by children over 5 years was 1·6 pt. in the two working classes and about 4·5 pt. in the two professional classes. There was a progressive reduction in the consumption of tinned milk by adults from 0·9 pt. 'milk equivalent' in the lower working to 0·1 pt. 'milk equivalent' in the lower professional class, while for children under 5 years consumption was relatively high at 1·1 pt.

Table 8. *Results of statistical analysis showing milk consumption according to age*

Social class ...		Lower working	Working	Lower professional	Professional
		Pints, liquid 'milk equivalents', per head per week			
Adults:	Liquid	1·6	3·5	4·3	4·6
	Tinned	0·9	0·3	0·1	0·1
	Dried	0·1	0	0	0
Children under 5 yr.:	Liquid	1·6	3·5	4·3	4·6
	Tinned	1·1	0	0·1	0·1
	Dried	0·1	0	0	0
Children 5–14 yr.:	Liquid	1·6	1·6	4·3	4·6
	Tinned	0·8	0·8	0·1	0·1
	Dried	0·1	0	0	0

Table 9. *Total milk consumption according to age, per head per week*

Social class	Adults consumption pt.	5–14 years		Under 5 years	
		Consumption pt.	% required	Consumption pt.	% required
Lower working	2·6	3·9	56	2·8	27
Working	3·8	3·7	53	3·5	33
Lower professional	4·4	5·7	81	4·4	42
Professional	4·7	5·8	83	4·7	45

'milk equivalent' among the lower working classes, but negligible in the other classes. For children aged 5–14 years, consumption was about the same at 0·8 pt. 'milk equivalent' in the working classes and negligible in the other classes. Too much precision must not be attached to the above figures, which should be taken as indications of the average intakes.

Table 9 shows the average total milk consumptions, as liquid milk, for the three age groups considered. The table also shows the percentage these intakes represent of adequate milk consumptions which are taken to be 10½ and 7 pt. per week for children under and over 5 years of age respectively.

On the average, the children of no social class consumed their full milk requirements, although those aged 5–14 years of the professional classes nearly did so. In all groups, children over 5 years had more of their requirements than did those under 5 years. Among the latter, and especially among lower working and working class families, consumption was very inadequate.

Discussion

The lower working class sample of the present study had the same definition as the slum families studied in 1936(4). In both studies it was found that the consumption of liquid milk was independent of family constitution, and that the consumption of tinned milk increased by about the order of $\frac{3}{4}$ pt. 'milk equivalent' per head per week for every additional person in the family, but was independent of changes in family housekeeping or food expenditure. In the 1936 study, liquid milk consumption rose by 1 pt. for every 14*s*. 7*d*. increase in housekeeping expenditure and every 9*s*. 6*d*. increase in food expenditure, the corresponding figures for the present study being 13*s*. and 8*s*. respectively. The differences, however, between these two sets of findings are not significant. Consequently, the results from the two studies are in close agreement.

The foregoing analyses reveal no differences between the four social groups concerning the uptake of school milk, although the reasons given for non-participation in the scheme are different for the various groups. There are wide differences, however, in respect of the consumption of liquid milk at home, the most interesting and important of which are the effect of changing family constitution on consumption. Lower working and working class families did not distribute the family milk supply among its members according to nutritional needs, but the lower professional and professional classes did so to a much greater extent. Changes in food expenditure had a greater effect on milk consumption among professional class families than among others. Perhaps the most interesting feature of the analyses of the factors influencing the consumption of tinned milk was that among lower working and working class families (no analyses being made for the other classes) neither housekeeping nor food expenditure affected consumption. In the former class, consumption was dependent on the number of persons in the family, but this was not so for the latter class. The four social classes differed in the proportions of the various types of tinned milk they consumed, the uses to which it was put and the reasons for purchase. Had the data for the four groups been analysed together, it is likely that it would have been found that tinned milk consumption fell with increasing food expenditures, but this would have been due to the fact that it so happened that food expenditure was higher and tinned milk consumption lower in the professional than in the working classes. The evidence from the analyses presented in this study shows that food expenditure had no effect on tinned milk consumption, the differences between the social classes being due to inherent social factors.

PART II. THE USES OF LIQUID MILK

The total liquid milk consumed in the four social groups was divided into that taken neat, in milk drinks, in cooking and breakfast foods, and in tea, coffee and cocoa. The percentages of the total milk supply used for these purposes are shown in Table 10.

Table 10. *Utilization of milk for various purposes, percentage of total*

Social class	Neat milk	Milk drinks	Cooking and breakfast foods	Tea, coffee and cocoa
Lower working	24	8	44	24
Working	15	8	44	33
Lower professional	17	12	35	36
Professional	25	10	34	31

Although for all social classes, in general, most milk was used in cooking, followed by that used in tea, etc., then by that drunk neat and finally that used in milk drinks, the distributions between these four uses varied for the four social classes. Thus, among lower working class families, 44 % was used in cooking and 24 % both for tea and drunk neat, but among professional class families the percentages were 34, 31 and 25 respectively.

FACTORS RESPONSIBLE FOR THE VARIATION IN LIQUID MILK CONSUMPTION BETWEEN THE SOCIAL CLASSES

The differences in average liquid milk consumption between the four social classes were due, so far as actual usage was concerned, to three causes:

(1) the percentage of families using milk for various purposes,

(2) the number of times per week that the various milk dishes or drinks were taken,

(3) the amount of milk used, per person per time, for the various milk dishes and drinks.

The data were analysed to provide information on these three aspects. The results are given in Table 11. The figures in columns B and C are not the average for *all* families of each social class, but that for the families *actually using* milk for the various purposes.

Table 11. *Percentage of households, etc., using milk for various purposes*

	Neat milk						Milk drinks					
	Adults			Children			Adults			Children		
Social class	A	B	C	A	B	C	A	B	C	A	B	C
Lower working	12	4·0	0·42	28	4·2	0·45	6	4·2	0·34	8	4·4	0·37
Working	21	2·8	0·40	32	5·1	0·39	15	2·7	0·27	16	3·9	0·24
Lower professional	24	3·2	0·48	67	4·2	0·59	38	2·3	0·26	26	4·8	0·40
Professional	31	3·4	0·50	81	4·7	0·60	37	2·0	0·28	24	5·4	0·41

	Puddings											
	Milk			Other			Custard			Baking		
Social class	A	B	C	A	B	C	A	B	C	A	B	C
Lower working	48	2·1	0·24	51	1·9	0·17	28	1·3	0·21	9	1·8	0·15
Working	70	2·6	0·27	82	2·4	0·16	42	1·6	0·27	21	1·1	0·19
Lower professional	65	2·4	0·33	72	2·4	0·15	73	2·3	0·21	43	1·1	0·18
Professional	64	2·4	0·29	72	2·5	0·16	67	2·2	0·19	40	1·2	0·19

	Sauce			Porridge			Other breakfast foods			Tea, etc.
Social class	A	B	C	A	B	C	A	B	C	A
Lower working	2	1·0	0·19	5	6·3	0·19	6	6·8	0·25	43
Working	15	1·2	0·17	10	5·8	0·25	15	4·6	0·26	79
Lower professional	38	1·9	0·13	16	5·5	0·14	36	4·9	0·21	98
Professional	56	2·0	0·13	15	6·2	0·18	36	5·8	0·19	100

Column A = percentage of families (or adults and children) using milk for the purpose.
Column B = no. of times per week milk dish made.
Column C = amount of milk used per person per time.

(1) *The percentage of families using milk for various purposes (Table 11, columns A)*

(*a*) There was an increase from the lower working to the professional class in the percentage of adults and children taking neat milk. There was a similar trend for milk drinks, except that the percentages are about the same for the professional classes. Even

in the professional classes only a maximum of 38 % of adults drank neat milk or milk drinks. 81 % of children of the professional classes drank neat milk.

(*b*) Milk and other puddings were made by 48–51 % of lower working class families, and by 64–82 % of families in the other classes. Only 28 % of lower working class families made custard, this increasing to 67–73 % for the professional classes.

(*c*) 5–9 % of lower working class families used milk for baking, porridge and other breakfast foods. Of the lower professional and professional families, however, 40–43 % used milk for baking, 15–16 % for porridge and 36 % for other breakfast foods. There was a great increase in the percentage of families using milk for sauce from the lower working to the professional classes.

(*d*) Almost all lower professional and professional class families used liquid milk for tea, coffee or cocoa, but only 43 % of lower working class families did so. Among them, tinned milk was frequently used for that purpose.

(2) *The number of times per week that the various milk dishes were eaten* (Table 11, *columns B*)

(*a*) No distinct differences can be discerned in the number of times per week that adults and children consumed neat milk, although the suggestion is that lower working class adults and working class and professional class children did so more frequently than others. There was a regular fall in the number of times per week that adults took milk drinks from the lower working to the professional classes, but children of the professional classes took them more frequently than those of the working classes.

(*b*) For milk and other puddings, the frequency was the same at 2·4–2·6 times for all except the lower working class, for whom it was 1·9–2·1 times. There was an increase in the frequency for custard and sauce from the lower working to the professional classes.

(*c*) Milk was used more frequently for baking among lower working class families than among others, and both porridge and breakfast cereals were eaten more frequently by the lower working and professional classes than by the working and lower professional classes.

(3) *Amount of milk used, per person per time, for the various milk dishes and drinks* (*Table* 11, *columns C*)

(*a*) Adults and children of the professional classes took somewhat more neat milk per time than did those of the working classes. Children of the professional classes took more in milk drinks than did children of the working classes.

(*b*) For milk and other puddings, custard and baking, the differences between the social classes were small. For sauce, porridge and other breakfast foods, the working classes tended to use more than the professional classes.

Milk used for soups, artificial cream and stewed fruit was not included in Table 11 because only very little was used for these purposes. Only up to 2 % of the families used milk for soups or stewed fruit, but 7 % of the professional class families did so for artificial cream.

Analysis was made to determine which milk drinks were most commonly taken by the families of the four social groups. Among all but the professional class, more milk was used in cocoa than any other drink, this being followed by Ovaltine. Among the professional class, most milk was used in coffee, followed by cocoa and then Ovaltine. Among

both the lower professional and professional classes more families made coffee than cocoa, but among all classes except the lower working almost the same number of families made Ovaltine as made cocoa.

THE USE OF MILK ON SUNDAYS AND WEEKDAYS

The milk consumption data were not analysed to show consumption on the different days of the week, because a separate study was made of that aspect. The data were, however, analysed to show the use of milk for cooking and for breakfast foods on Sundays and weekdays. The amount of milk used for these purposes on Sunday and weekday is shown in Table 12. The table shows that there was an increased use of milk for cooking and breakfast cereals among the working classes, but a reduction among the professional classes.

Table 12. *Milk used on Sundays and weekdays for cooking and breakfast foods*

Social class	Per weekday pt.	Sunday pt.
Lower working	31	90
Working	81	108
Lower professional	91	61
Professional	122	91

The data were analysed further to demonstrate how frequently various dishes were eaten on Sundays compared with weekdays. The findings can be summarized as follows. Among lower working class families, the greater amount of milk used in cooking on Sundays was due to the increased consumption of milk and other puddings and custard. Among working class families, it was due to increased consumption of 'other' puddings and custards, although there was a reduction in the use of milk for sauce, baking, porridge, and other breakfast cereals. Among the lower professional class and the professional class families, there was a general reduction in the use of milk for milk dishes except 'other puddings', for which there was an increase. Thus among lower working class families there was an increase in the uses to which milk was put on Sundays compared with weekdays; the reverse was so for the professional classes. The working and lower professional classes fell between the two extremes.

DISCUSSION

The foregoing analyses show that there are considerable differences in the utilization of milk between the four social classes of consumers. Perhaps the principal difference is in the percentage of families using milk for various purposes, but there are also important differences both in the frequency with which milk is used for some purposes and the amount used per person per time.

These social class differences are masked when the data of all social classes are analysed together (Murray (1); Murray & Rutherford (2)). It is not possible, however, to determine from the present study whether the differences between the social classes are due to inherent social characteristics or to the differences in the amount of milk consumed. The problem can best be put in the form of the question: 'If lower working class families had the same amount of milk as professional class families, would it be used in the same way in both classes?' But the impression has been formed as the result of actual contact with families of different social classes that milk consumption among poorer families is held back not only by lack of purchasing power but also by lack of cooking and other facilities

and lack of knowledge concerning the uses to which milk can be put. Further differences in household routine affect not only total consumption but also the use of the available milk. It is highly probable that inherent social factors do influence, to a marked degree, the utilization of milk by different social classes of consumers.

PART III. THE CAPACITY TO PURCHASE MILK

The data collected during the survey enabled a quantitative estimate to be made of the number of families in the four social classes who purchased and who could not afford to purchase an adequate amount of milk.

Relationship of the actual to the adequate consumption of milk

The minimum milk requirements used for the analysis were those suggested by George (8), with the addition that a child of under 1 year of age was allowed $8\frac{3}{4}$ pt. per week. These are given in Table 13.

Table 13. *Standards of milk requirements*

Age	Amount
Child below 2 years	$8\frac{3}{4}$ pt. per week
Child from 2–5 years	$10\frac{1}{2}$ pt. per week
Child from 6–14 years	7 pt. per week
Adult	$3\frac{1}{2}$ pt. per week

The total milk consumption of each family was the sum of liquid, tinned and dried milk, the milk consumed in school being included in the total. The milk requirement of each family was calculated from its constitution and the requirements given in Table 13. Table 14 shows the number of families and persons in three age groups consuming various

Table 14. *Percentage milk requirements consumed*

Social class ...		Lower working				Working		
		Children				Children		
% milk requirements	A.	0–5	5–14	F.	A.	0–5	5–14	F.
0– 25	11	4	5	6	1	0	0	1
25– 50	96	36	29	40	57	11	22	22
50– 75	115	12	24	51	129	12	29	44
75–100	54	5	9	25	108	5	13	39
100 or more	54	1	3	28	122	0	2	46
Total	330	58	70	150	417	28	66	152

Social class ...		Lower professional				Professional		
		Children				Children		
% milk requirements	A.	0–5	5–14	F.	A.	0–5	5–14	F.
0– 25	0	0	0	0	0	0	0	0
25– 50	2	1	0	1	0	0	0	0
50– 75	38	8	3	14	31	6	6	11
75–100	62	6	8	17	80	10	19	26
100 or more	257	5	8	92	306	7	26	98
Total	359	20	19	124	417	23	51	135

A. = adults; F. = families.

proportions of standard milk requirements. Of the lower working, working, lower professional and professional class families, 19, 30, 74 and 78 % took their full milk requirements. 31 % of lower working class families and 15 % working class families took less than 50 % of their milk requirements, these families containing 69 and 40 % of

children under 5 years of age and 49 and 33 % of children over 5 years of age respectively. Almost no children of the lower working and working classes belonged to families consuming their full requirement; for the lower professional and professional classes, 25 and 30 % respectively of children below 5 years and 42 and 50 % respectively of those between 5 and 14 years belonged to families consuming a proper amount of milk.

The capacity to purchase milk. An estimate was made of the extent to which underconsumption is due to lack of purchasing power.

The technique employed in this present analysis is a development of that used previously (Bransby (4)). Each person was allocated, according to age, a minimum standard food expenditure sufficient to obtain a diet adequate for health, according to the method suggested by George (8). These diets were the B.M.A. no. 2 diets (9), adjusted to allow larger milk consumptions. Adjustment was made for the difference for the retail cost of food in June 1939. The food costs allowed were:

	Up to 2 yr.	2–3 yr.	3–6 yr.	6–8 yr.	8–10 yr.	10–12 yr.	12–14 yr.	Adult
	s. d.	*s. d.*	*s. d.*	*s. d.*	*s. d.*	*s. d.*	*s. d.*	*s. d.*
With milk	3 3	4 0½	4 5¾	4 9¼	5 4½	6 0¾	6 9	7 2
Without milk	1 0¾	1 5	1 10¼	3 0¼	3 7½	4 3¾	5 0	6 3½

The actual food expenditure of each family was then compared with the standard family expenditure, both including and excluding milk, determined from the family constitution and the above allowances. If the actual expenditure fell below the standard expenditure *including* milk, the family could not purchase the desirable milk ration at full liquid price. In that case the standard expenditure *excluding* milk was subtracted from the actual expenditure, the difference being the money available for the purchase of the desirable milk allowance. This sum divided by the milk allowance gave the price per pint at which the family must purchase milk to afford the proper amount. This technique depends on the assumption that the actual food expenditure of the family is the highest it can reasonably afford. This may not be true on theoretical grounds, as consumers in general have not sufficient knowledge to spend their available money to obtain the greatest nutritional advantage from it. But it is, at least, the food expenditure which fits in best with the household economy and prevailing food habits.

Table 15 shows the price at which the populations of the groups must purchase milk in order to be able to buy the full allowances. 47 % of the lower working class and 28 % of the working class families did not spend sufficient to purchase sufficient milk at full price, but only one family of the lower professional and professional classes came into this category. These lower working and working class families contained respectively 59 and 40 % of the children in the classes under 5 years and 67 and 54 % of those between 5 and 14 years of age. About 50 % of the lower working and working classes who could not afford the full amount of milk could make no contribution towards its cost.

This analysis also indicates that some families spent sufficient on food to purchase their full allowance of milk, even though they did not do so. For example, 81 % of lower working class families consumed less than the desirable amount, but only 47 % were not in a financial position to do so, while among the lower professional and professional classes, only 1 % of the 26–27 % who did not purchase the full amount, could not afford it. There was thus scope for education and propaganda to encourage consumers to purchase milk to the limit of their ability.

Table 15. *Price at which milk must be purchased to obtain full allowance*

Social class ...	Lower working class				Working class			
		Children				Children		
Price per pint	A.	0–5	5–14	F.	A.	0–5	5–14	F.
Free	69	9	22	35	51	2	15	21
Up to 1*d.*	35	12	9	15	18	2	4	7
1*d.*–2*d.*	14	8	5	7	25	5	10	8
2*d.*–3*d.*	31	5	11	13	24	2	7	7
Full price	181	24	23	80	299	17	30	109
Total all families	330	58	70	150	417	28	66	152
Social class ...	Lower professional class				Professional class			
		Children				Children		
Price per pint	A.	0–5	5–14	F.	A.	0–5	5–14	F.
Free	0	0	0	0	0	0	0	0
Up to 1*d.*	0	0	0	0	3	0	1	1
1*d.*–2*d.*	0	0	0	0	0	0	0	0
2*d.*–3*d.*	5	0	1	1	0	0	0	0
Full price	354	20	18	123	414	23	50	134
Total all families	359	20	19	124	417	23	51	135

A. = adults; F. = families.

GENERAL SUMMARY

From the data included in Parts I, II and III, the following word pictures describe the state of milk consumption among the four social classes.

Lower working class. Among lower working class families, the attitude towards liquid milk, although positive, was not sufficiently active to bring about any considerable consumption of liquid milk. On the other hand, the attitude towards tinned milk was such that it formed the basis of the family milk supply. The average consumption of liquid milk was 1·58 pt. and tinned milk 0·91 pt. 'milk equivalent' per head weekly. The consumption of liquid milk was independent of family constitution, but that of tinned milk increased by about $\frac{3}{4}$ pt. for every additional person in the family. The consumption of liquid milk increased with increases in housekeeping expenditure and food expenditure, but that of tinned milk did not. The available milk supply was not distributed among the members of the family according to nutritional needs.

Less than half the families used liquid milk in tea, tinned milk being used for this purpose. Coffee, cocoa and other milk drinks were seldom drunk. About one-quarter of the children and one-eighth of the adults drank neat milk at home at the rate of $1\frac{1}{2}$–2 pt. per week. 44 % of the total liquid milk supply was used for cooking and breakfast foods, the most popular dishes being 'other' puddings, milk puddings and custard. Milk was seldom used for any other purposes, such purposes accounting for only 9 % of the total milk supply. More milk was used for cooking and breakfast foods on Sundays than on weekdays, due mainly to a greater consumption of milk and 'other' puddings and custards.

The low milk consumption was to some extent due to inadequate food expenditures. 81 % of the families did not purchase an adequate amount of milk, these families containing practically all the children in the sample. 49 % of the families, containing 59 and 57 % of children under and over 5 years of age respectively, did not spend enough on food to purchase their proper amount of milk. Even among the families studied there was room for a greater consumption of milk within the range of prevailing incomes and the prevailing price of milk.

Working class. The attitude towards liquid milk was much more active than among lower working class families, and liquid milk formed the basis of the family milk supply. Consumption of liquid milk rose to 2·61 pt. but that of tinned milk fell to 0·42 pt. 'milk equivalent' per head weekly. The tinned milk was used almost entirely in tea, but 79 % of the families used liquid milk in tea, coffee or cocoa. Liquid milk consumption increased by $1\frac{3}{4}$ pt. weekly for every additional adult in the family, but did not increase with additional children, while that of tinned milk was independent of the number of both adults and children in the family. The family milk supply was not distributed among the members of the family according to nutritional needs. The consumption of liquid milk increased with increases in housekeeping and food expenditures but tinned milk did not. Somewhat more adults and children than among lower working class families drank neat milk or milk drinks, the former at the rate of 1–2 pt. and the latter about 1 pt. per week. 44 % of the total milk supply was used for cooking and breakfast foods, the most popular dishes being 'other' puddings, followed by milk puddings and custards. More milk than among lower working class families was used for baking, sauces and breakfast cereals. There was a greater use of milk for cooking on Sundays than on weekdays, due mainly to an increased consumption of 'other' puddings and custard.

Compared with the lower working class families, the greater average consumption of liquid milk by working class families was due mainly to a greater proportion of the families using milk for the various purposes, especially milk in tea and in milk and other puddings and, to a lesser extent, as neat milk, and in milk drinks and custards.

The consumption of liquid milk was to some extent restricted by lack of purchasing power. 70 % of the families did not purchase an adequate amount of milk, and 28 % of the families did not spend enough on food to enable them to do so. The families not purchasing sufficient milk contained practically all the children in the sample.

Lower professional class. The attitude towards liquid milk was sufficiently active to bring about a considerable increase in consumption compared with the working classes. Liquid milk consumption was 4·32 pt. per head weekly, that of tinned milk being negligible. Liquid milk consumption rose by $1\frac{3}{4}$ pt. and about 4 pt. for every additional adult and child respectively in the family, indicating that milk was distributed to some extent among the members of the family according to nutritional needs. Consumption also increased with increased food expenditure.

24–38 % of adults drank neat milk and milk drinks at the rate of $1\frac{1}{2}$ and $\frac{1}{2}$ pt. per week respectively, but 67 % of the children drank neat milk and 26 % milk drinks at the rate of 2–$2\frac{1}{2}$ pt. per week. 35 % of the total milk supply was used for cooking and breakfast cereals, the most popular dishes being 'other' puddings, milk puddings and custard. Slightly less families than among the working class made milk and 'other' puddings, but considerably more used milk for other purposes. Less milk was used for cooking on Sundays than weekdays due to a general reduction in the consumption of milk dishes except 'other' puddings. The greater average consumption of liquid milk by the lower professional class compared with working class families was due mainly to the increase in the number of families making the various dishes, although the greater consumption of neat milk and milk drinks by children made a useful contribution.

All families (except one) spent enough money on food to be able to purchase an adequate amount of milk, but 26 % of the families did not do so. There was thus scope for increased consumption at the prevailing price of milk.

Professional class. Taken generally, the differences between the lower professional and professional classes were not marked. Consumption of liquid milk rose to 4·91 pt. and that of tinned milk to 0·14 pt. 'milk equivalent' per head weekly, the latter being used for cooking or special purposes. Liquid milk consumption changed in the same way as among lower professional class families with changes in family constitution, but changed to a greater extent with changes in food expenditure. 31 %, that is, slightly less than among lower professional class families, of the liquid milk supply was used for cooking and breakfast cereals, but apart from a small increase in the use of milk for sauce and artificial cream, the popularity of the various milk dishes was about the same as for the lower professional class. Slightly more adults and children took neat milk, and slightly more per week, than among lower professional class families, and this was the main cause of the differences in consumption between the two groups. Less milk was used for cooking on Sundays than on weekdays, due to the same causes as for the lower professional class. All families spent enough on food to purchase an adequate amount of milk, but 22 % did not purchase their proper allowance. With the prevailing incomes and price of milk, there was thus scope for an increased consumption of liquid milk.

The present study was confined to one locality, namely, the city of Leeds, and it cannot be assumed that the results obtained apply to the population in other parts of the country. The object of the study was to demonstrate the differences in milk consumption habits of different social classes of consumers, and the results obtained justify the conclusion that the differences are so fundamental that in any investigation into the consumption of milk it is essential to study the various social classes separately. If a sample of consumers of various social classes is studied, the 'average' results for the sample mask important differences between the different social groups.

SUMMARY

1. Data on milk consumption, and other relevant information, were obtained from 150, 152, 124 and 135 lower working, working, lower professional and professional class families respectively in the city of Leeds during May–June 1939.

2. An account is given of the factors affecting the consumption of liquid milk at school and at home, and of tinned milk. An estimate is made of the amount of milk consumed by children over and under 5 years of age and by adults, and of the nutritional adequacy of the milk consumption of those three groups.

3. An analysis is made of the use of milk in the home and the importance of the three factors: (*a*) the number of families making milk dishes, (*b*) the frequency with which the dishes are made, and (*c*) the amount used in each dish per person per time, in causing differences in the average liquid milk consumption of the four social classes.

4. An estimate is made of the extent to which families were obtaining a proper amount of milk, and the number of the families who were not spending sufficient on food to purchase an adequate amount of milk.

5. It is concluded that in investigations into the consumption of milk, the various social classes should be studied separately, otherwise fundamental differences between them fail to be disclosed.

My thanks are due to the Agricultural Economics Research Institute, Oxford, for financing this study. It is a pleasure to record my appreciation of the work of Miss R. M. Hartley, Miss G. M. Jackson and Mrs N. B. Bransby (Miss Leigh Smith), who collected the data, and to Dr H. E. Daniels for assistance and guidance in connexion with the statistical aspects of the investigation.

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340. VARIATIONS IN THE DEMAND FOR MILK AND CREAM AMONG FOUR SOCIAL CLASSES OF CONSUMERS IN THE CITY OF LEEDS

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(With 4 Figures)

The general experience of the retail milk trade is that considerable variations exist in the demand for milk and cream at different times of the year, and that these need careful consideration to preserve the proper balance between the supply of, and demand for milk. Different social classes of consumers are also known to exhibit different habits of milk and cream consumption. This paper examines the fluctuations of demand for milk and cream over a 1-year period of four social classes of consumers.

TECHNIQUE

The investigation was undertaken in the city of Leeds, and districts for investigation were chosen by inspection as representative of the four social classes under examination:

Lower working class. All the houses were condemned as unfit for human habitation. Householders followed unskilled occupations.

Working class. The houses were not condemned. The householders followed more skilled occupations than did those of the lower working class.

Lower professional class. Typical householders were clerks, insurance agents and commercial travellers.

Professional class. Householders held higher grade appointments such as University staff, barristers and company directors.

The milk sales of a number of milk rounds of the Leeds Industrial Co-operative Society Ltd. serving the selected districts were analysed for the 1-year period from the week ending 3 September 1938 to the week ending 26 August 1939. These rounds comprised about 400–600 customers for each social class. The number of customers in any round varied from time to time. Therefore, to obtain a working basis for the year, the number of customers in each round was counted weekly, rounds serving the same social class were combined, and the weekly milk and cream sales of the combined rounds were divided by the number of customers in the combined rounds. It is the fluctuations in demand *per* 100 *customers* which are considered in this report. No data were available to determine the average size of the households served, and therefore no 'per head' information is included.

No allowance was made for milk and cream which may have been purchased from other retailers. For milk, it is probable that these additional purchases amounted to but a small proportion of the recorded sales, and there is no reason to suppose that fluctuations in the demand of consumers purchasing milk from more than one milk retailer are different from those of consumers who have only one supplier. In some parts of the country, it is the custom to purchase cream to some considerable extent from shops, but in the north of England, including the city of Leeds, few shops sell cream. It is probable that the demand for cream and milk as recorded in the following analysis is reasonably representative of the districts and social classes under review.

The demand for milk

The average milk sales throughout the year, per 100 customers per week, was 613, 623, 1029 and 1109 pt. among lower working, working, lower professional and professional families respectively. The sales of certified milk to ordinary milk was in the proportion of 1 to 132, 124, 139 and 38 among the same four social classes respectively.

Weekly variations. Fig. 1 shows the weekly variations in demand throughout the year. The range of fluctuation was 15, 13, 16 and 22% in the lower working, working, lower professional and professional classes respectively, suggesting a lesser rigidity of consumption among the professional class families than among those of other social classes.

Fig. 1 shows that holiday weeks were times of reduced demand, but that between holidays, demand tended to increase somewhat to a peak and then fall. These peaks and troughs broke up the general trend of demand throughout the year. For all social groups, the year can be divided into two periods—from the beginning of September to about the beginning of July and thence to the end of August. For all classes the former period was one of slightly increasing demand, that at the beginning of July being about 11, 4, 8 and 14% greater than at the beginning of the previous September among lower working, working, lower professional and professional class families. In the second period, demand fell to slightly above or below the level of the previous September. This falling demand was probably due to the warmer weather.

Daily variations. The total sales for each day of the week throughout the year were determined for each social class. Demand on Sunday was higher than on weekdays;

Table 1 shows the demand on each day of the week expressed as a percentage of that on Sunday. Table 2 is expressed graphically in Fig. 2.

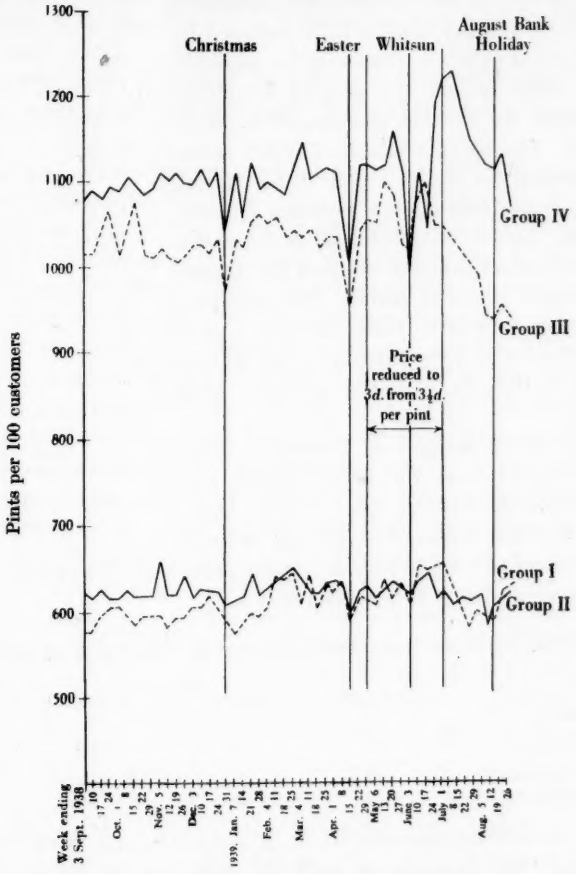

Fig. 1. Weekly fluctuations in milk sales per 100 customers.

In Figs. 1–4, Group IV = Professional class. Group III = Lower professional class. Group II = Working class. Group I = Lower working class.

The fall in demand from Sunday to Monday was 9, 16, 37 and 41% for the lower working, working, lower professional and professional classes respectively. In all groups

Table 1. *Daily variations in the demand for milk*

Social class	Sun.	Mon.	Tues.	Wed.	Thurs.	Fri.	Sat.
Lower working	100	58·7	61·8	60·2	61·0	57·9	59·4
Working	100	63·0	67·3	65·7	67·6	62·1	64·5
Lower professional	100	84·1	87·5	87·8	88·2	87·0	87·5
Professional	100	91·1	93·2	93·8	93·4	94·0	94·8

demand increased by 2–4% on Tuesday. It then remained for the rest of the week at the Tuesday level for the lower professional and professional classes. Among the lower working and working classes it remained at the Tuesday level until Thursday, then fell

a further 4–5% on Friday with a small recovery on Saturday. The differences in the daily variations of demand among the four social classes were due mainly to differences in food-consuming habits, domestic routine and purchasing power. Among working class families milk was used for cooking only to a small extent on weekdays but generally so on Sundays, while among the professional classes milk was used both for cooking and breakfast cereals through the week. The fall in demand on Friday among working class families was due to Friday being 'turning out day' with the subsequent upset of domestic routine.

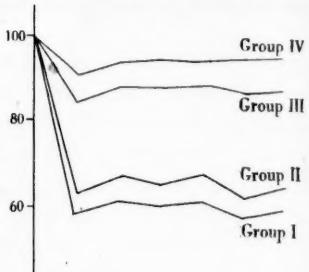

Fig. 2. Daily fluctuations in milk sales.

Holiday periods. Table 2 shows the fall in demand at holiday periods expressed as a percentage of the demand during an immediate previous period. The Christmas week demand was compared with that of 1 week previous, Easter week with 2 weeks previous and Whitsun and August Bank Holiday with the previous 'peak' demands.

The percentage fall in demand at Christmas, Easter and Whitsun increased from the lower working class to the professional class, but this was not so at August Bank Holiday. Weather conditions obviously influence the change in demand at holiday periods, except probably at Christmas. At all holidays, except August Bank Holiday, the weather was fine.

Table 2. *Percentage reduction of demand at holiday periods*

Social class	Christmas %	Easter %	Whitsun %	August Bank Holiday %
Lower working	1·8	5·3	3·5	11·3
Working	2·4	7·2	3·1	6·6
Lower professional	5·7	7·8	7·4	15·0
Professional	6·5	10·1	14·6	9·6

Analyses were made to determine on which days of the holiday weeks demand was most markedly affected. The demands on each day from the Friday previous up to the Thursday following the holiday were compared with the average demand on the 4 corresponding days of the 2 weeks previous to and 2 weeks following the holiday. At Easter and Whitsun, demand on the Friday previous to the holiday fell among the professional classes by 3–8%, but among the lower working and working classes it rose by 3–5% at Easter. At Easter and Whitsun, demand fell on the Saturday by up to 6% among the lower working class and by 9–20% among the professional classes; on Sunday and Monday there was a further fall of up to 9% in all classes. There was partial recovery among all but the lower working class on Tuesday; for that class there was a further fall of 2–5% with recovery on Wednesday almost to the pre-holiday level. For the other classes, recovery of demand is slower than for the lower working class. At August Bank Holiday, demand fell by 1–4% among the working classes and 6–7% among the professional classes on Friday, and by 11–22% on Saturday. The Sunday, that is, August Bank Holiday Sunday, was wet, and demand recovered to almost normal, but fell by 4–17% on Monday and Tuesday, the greatest fall being among working class families.

By Wednesday, recovery to normal had almost been achieved in all but the lower working class. At Christmas there was little change in demand up to Christmas Day, except among the professional class, among whom from Christmas Eve until the Wednesday following Christmas it fell by about 10%. Demand fell by 8% among the lower working class, but only 12% among working class families on Boxing Day. Among all classes demand was 7–12% below normal on Tuesday. By Wednesday recovery was almost complete among lower working class families, but was still 6–8% below normal in the other classes.

The fall in demand at Christmas extended over 1 week among the professional classes and the working class and 2 weeks among the lower working class. At Easter, demand recovered its previous level in 1 week among professional class families, 2 weeks among working class and 4 weeks among lower working class families. At Whitsun, the fall in demand was recovered in 1 week among all classes except the lower working class for whom it was somewhat prolonged. The fall in demand at August Bank Holiday was the culmination of a fall in demand over several weeks, and it seems probable that the fall, actually due to the holiday, was not so marked as for other holidays.

The above analyses show that for holiday periods there was generally a greater reduction among the professional than among the working classes, and that there was a tendency for the reduction to commence among the former on the Friday and among the latter on the Saturday preceding the holiday. Except at Christmas, demand was generally lowest on the Monday of the holiday periods, except for lower professional class families when it occurred on Tuesday. Demand had recovered more by the week-end following the holiday among the working than among the professional classes except at August Bank Holiday. It is customary for a large number of working class persons to take their holidays during August Bank Holiday week, and it is likely that this is the cause of the difference in the recovery at August compared with the other holidays. The effect of the holiday period as reflected in a fall in demand is spread over a longer period among the working than among the professional classes. This may be due to lack of purchasing power among the working classes because of the heavy spending during the holidays.

Seasonal variation. The average weekly consumption was determined for each social class for the seasons October–December, January–March, April–June, and July–September. Table 3 gives these averages, expressed as percentages of the highest seasonal demand of each social class.

Table 3. *Seasonal variations in the demand for liquid milk*

Social class	Oct.–Dec.	Jan.–Mar.	Apr.–June	July–Sept.
Lower working	96	98	100	98
Working	99	100	99	98
Lower professional	98	100	100	96
Professional	97	98	97	100

In the interpretation of these figures, it must be borne in mind that the season October–December includes Christmas, April–June includes Easter and Whitsun and July–September includes August Bank Holiday. The seasonal variations are small for all social classes, the greatest amounting only to 4%.

The effect of price change on consumption. The price of ordinary, as distinct from certified, milk was 3½*d.* per pint except for the 2 months 30 April to 1 July, when it was 3*d.* The

cheaper milk period occurred during a period of considerable fluctuations in demand, including that due to Whitsun. The price reduction to 3*d.* took place at a time of rising demand, and the price was increased again to 3½*d.* when demand was falling. The weekly demand figures showed no changes which could be attributed to the price changes.

The demand for cream

Weekly variations. Fig. 3 shows the weekly demand for cream throughout the year for each of the four social classes. There was a very marked peak for each class at Christmas. Apart from this Christmas peak, demand did not exceed about 30*d.* per 100 householders

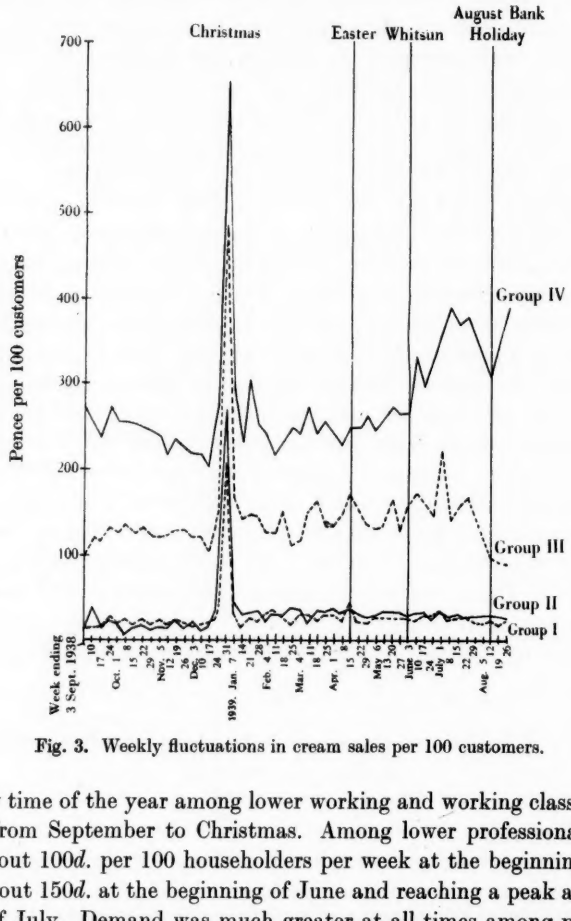

Fig. 3. Weekly fluctuations in cream sales per 100 customers.

per week at any time of the year among lower working and working class families, falling to about 20*d.* from September to Christmas. Among lower professional class families, demand was about 100*d.* per 100 householders per week at the beginning of September, increasing to about 150*d.* at the beginning of June and reaching a peak at 200*d.* at about the beginning of July. Demand was much greater at all times among professional class families than among others. At the beginning of September it was about 270*d.* per 100 families per week, falling to just over 200*d.* at the middle of December, increasing to about 250*d.* at the beginning of June, and 380*d.* at the beginning of July.

Daily variations. The same technique was followed as in the corresponding analyses for milk in the examination of the daily variations in the demand for cream. Table 4 shows

the average cream sales for each day of the week throughout the year expressed as a percentage of the Sunday sales. Table 4 is expressed diagrammatically in Fig. 4.

Table 4. *Daily variations in the demand for cream*

Social class	Sun.	Mon.	Tues.	Wed.	Thurs.	Fri.	Sat.
Lower working	100	10·9	6·4	8·2	6·1	12·2	6·5
Working	100	10·0	2·2	1·9	2·2	5·4	10·6
Lower professional	100	6·2	6·7	7·3	6·1	6·9	13·0
Professional	100	15·9	18·7	21·0	18·0	16·1	38·9

The demand for cream on Sundays far exceeded that on other days. Considering the low actual demand by lower working and working class families, cream sales among these families on weekdays were almost negligible, while those among lower professional class families were small. For lower professional and professional class families, daily demand on Monday to Friday was 6–7 and 16–21% respectively of Sunday demand, these doubling on Saturday. Among working class families demand fell to 10% of the Sunday sales on Monday, and then to 2% on Tuesday to Thursday, increasing to 5 and 11% on Friday and Saturday respectively. Among lower working class families, demand fell to 10% on Monday and then remained at 6–8%, except on Friday, when it rose to 12%. The above analyses show that cream was very much a Sunday luxury among all social classes.

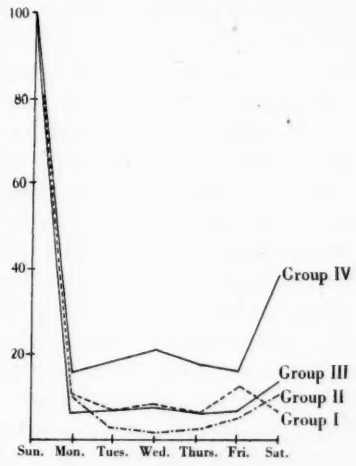

Fig. 4. Daily variations in cream sales.

Variations due to Bank Holiday periods. The only holiday period which had any noticeable effect on the demand for cream was Christmas. The percentage increases during the Christmas week over those of a fortnight previous were 1480, 1570, 360 and 230% among lower working, working, lower professional and professional class families respectively. The increase during the Christmas week takes place mainly on Christmas Eve, Christmas Day and Boxing Day. There was also some increase on New Year's Day.

Seasonal variations. Table 5 shows the average weekly cream sales expressed as percentages of the average sales during the seasons of greatest demand, for each of the four social classes, for the seasons October–December (excluding the week prior to and Christmas week), January–March, April–June and July–September.

Table 5. *Seasonal variations in the demand for cream*

Social class	Oct.–Dec.	Jan.–Mar.	Apr.–June	July–Sept.
Lower working	76	100	98	82
Working	46	100	95	79
Lower professional	77	94	100	90
Professional	71	77	87	100

There were considerable fluctuations in demand in the various seasons. For each social class, demand was least during October–December. The greatest demand was during January–March among lower working and working class families, during April–June

among lower professional class families, and during July–September among professional class families.

Discussion

Cohen(1), from a study of the milk sales of two milk retailers in four towns in the Midland area of England during December 1936 and 1937, found that sales on Sunday were 16% above the average daily sales, with a drop to 6% below average on Monday and recovery to 97–99% for the rest of the week. It was found that this Sunday peak always occurred in all of nine towns for which figures were obtained, but that the excess varied considerably. Thus, in a fairly prosperous country town in Yorkshire, sales on Sunday during December and January were 5% above the average, while in a depressed town in south Wales they were 41% greater. The daily variations found by Cohen(1) and in the present study are in broad agreement, for greater daily variations are found among the depressed areas and the working classes than among prosperous areas and the professional classes. Neither of the studies provides definite information as to whether the differences in daily fluctuations are due to differences in income or to inherent social class differences. The results of the present study did not confirm the findings for the changes in demand found by Cohen on Christmas Day. That study shows that sales on Christmas Day were higher than usual, varying from 6% in prosperous to 30% in depressed areas, but in the present study sales per 100 customers increased by 4% among lower working class families and decreased by 9% among professional class families. The methods of estimating changes in demand were of course different in the two studies, Cohen working on total sales, and the author in the present study on sales per 100 customers.

The shapes of the curves showing the weekly demand for milk throughout the year are more or less the same for the four social classes examined. This suggests that the factors which influence demand throughout the year do not affect one social class to a much greater extent than any other. But examination of Fig. 1 reveals that for each social class temporary spurts in demand occur which have no counterpart among the other social classes. No explanation of these temporary differences can be offered.

It is interesting to compare the demand for liquid milk found in a milk consumption study of the same social classes in Leeds during May–June 1939 (Bransby(2)) with that found in the present study over the same period. In that study the consumption of liquid milk was 533, 901, 1395 and 1800 pt. compared with about 600, 650, 1050 and 1200 pt. found in the present study for the lower working, working, lower professional and professional classes respectively. The consumptions found in the former study are generally higher than in the present study. The differences may be due to several factors. The milk purchases from other milk retailers in addition to the Co-operative Society may have been larger than anticipated; consumers obtaining milk from the Co-operative Society may not be representative of all consumers in the localities studied; there may have been overstatement of milk purchases in the family survey; and the sample studied in the family survey may have been too small to give representative results. No explanation of the differences can with certainty be given, but considering that only 49, 58, 43 and 13% of the milk supply of the lower working, working, lower professional and professional class families studied was pasteurized, and could thus have been obtained from the Co-operative Society, it is feasible that the samples examined in the two studies were not exactly comparable.

One point of general interest in survey work arises from examination of Fig. 1. The object of family surveys is to obtain information on the food habits of the sample studied. The most settled period of milk consumption is from the beginning of September to about the middle of December; all other periods are influenced by the peaks and troughs referred to previously. Consequently, unless a study is required at any particular time of the year, the period September–December is the most suitable for obtaining information on the consumption of milk.

Beilby(3) reported the results of a study of cream consumption in Oxford in 1938 and compared the results with those found in 1936 (Murray(4)). The studies were made in the summer of each of the two years. Expenditure on cream increased from 0·76*d.* in 1936 to 0·90*d.* in 1938 per head weekly. In 1938, expenditure was 328*d.* per 100 families per week. Demand on Sunday was 63% the total weekly demand, but demand on Saturday was not noticeably different from that on any other weekday. The families studied in the Oxford investigations were a random sample of Oxford families and thus contained families of different social classes. The demand for cream depends both upon the ability to purchase it and the incentive there is to do so. The incentive to use cream also depends on the ability to purchase such foods as soft fruit or tinned fruit with which cream is eaten. Among lower working and working class families, cream was purchased to a very limited extent throughout the year, and there was but a small actual increase in demand during the summer and soft-fruit season. The much smaller demand throughout the whole of the year, by working class compared with professional class families, shows that, in all probability, cream was too dear for them to purchase, and the strictly limited increase during the soft-fruit season shows that the incentive was insufficient, probably because of the cost of soft fruit. The demand by the lower professional class families was about half-way between that of the working and that of the professional class, and the increase during the summer lasted only a short while. It seems likely that among lower professional class families, the price of cream limited demand very considerably, and during the summer cream was only purchased to any increased extent when the price of soft fruit was low. Among professional class families, the summer increase lasted from almost the beginning of July to the beginning of September. Even among the professional class there was little demand for cream on weekdays, the greatest weekday demand being 39% on Saturday but only 21% on other days of the Sunday demands. Comparison of the average daily demand for cream among professional class families in the four seasons October–December, January–March, April–June, July–September, reveals that, on the average, there was only a 21% difference in demand on Sundays, but a 46–73% difference for the other days of the week. Demand on weekdays was more flexible than on Sundays. The fact that consumers purchased more cream, when the incentive was there to do so, shows that even at the prevailing price of cream the money was available for an increased purchase in those seasons when demand was low. It therefore seems that among professional class families there is room, at least during a considerable part of the year, for an increased demand for cream at prevailing prices. Among lower professional families price was a limiting factor. If the price were reduced demand would probably increase considerably. Among lower working and working class families, however, the demand for cream was so small that only a drastic reduction in price would be likely to affect demand.

My thanks are due to Mr E. E. Holmes, Dairy Manager of the Leeds Industrial Co-operative Society Ltd., for giving me facilities to undertake this work, and to his staff for their kindness and co-operation during the extraction of the data.

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341. THE EFFECT OF THE PRE-HEATING TEMPERATURE ON THE BACTERIAL COUNT AND STORAGE LIFE OF WHOLE MILK POWDER SPRAY-DRIED BY THE KRAUSE PROCESS

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(With 5 Figures)

INTRODUCTION

A few years ago a large-scale experiment was carried out by Mattick *et al.*(1) to determine the effect of the pre-heating temperature, of clarification and of the bacteriological quality of the raw milk on the storage life and bacterial count of full-cream milk powder. In that work the Kestner spray-drying principle was used, and the pre-heating treatments adopted were 165 and 190° F. for approximately 20 sec. Of the three main factors investigated, the pre-heating temperature was found to have the greatest influence on the keeping quality of the powder. Dried milk made from liquid milk which had been pre-heated to 190° F. kept in good condition at room temperature for two or three times as long as powder made from milk which was pre-heated to only 165° F. The longer lived powder had a slight but not unpleasant cooked flavour. The higher pre-heating temperature also resulted in the product having lower bacterial counts. It caused streptococci to predominate over micrococci and brought about a relative increase in the number of spore bearers.

The effect of the higher pre-heating temperature was obviously so great that plans were made for further experiments to find whether the pre-heating temperature was equally important for powder dried by other spray-drying processes and also to find what pre-heating temperature gave the maximum storage life with the minimum degree of 'cooked' flavour. The first series of experiments, which are described in the present paper, were made at a Krause drying plant. The general principles underlying the Krause process can readily be obtained by reference to publications by Hunziker(2) and Scott(3). As in the

Kestner process, the milk is sprayed by centrifugal force. The pre-heating temperatures investigated were 160, 170, 180, 190 and 200° F.

Through the courtesy and helpful co-operation of the Directors of Dried Milk Products Ltd., permission was obtained to use the Krause spray-drying plant at Northallerton for the present experiments. In a preliminary discussion help was given by Messrs H. C. Hillman, B. A. R. Wood and A. F. Shutter, of Dried Milk Products Ltd., Dr A. T. R. Mattick, of the National Institute for Research in Dairying, Reading, and Mr D. A. McKenzie, Advisory Bacteriologist for Yorkshire. Messrs Wood and Shutter kindly agreed to supervise the drying operations during the period of the experiment, while Mr McKenzie generously made available laboratory accommodation, equipment, media and technical assistance for the bacteriological tests which were carried out by Dr Higginbottom throughout the milk-drying period. To all those who so willingly helped in the initial discussion and during the part of the work carried out at Northallerton the authors tender their sincere thanks.

THE DRYING OF THE MILK

The milk was dried between 21 and 30 June 1943. The only alteration made in the normal working of the plant was that five different pre-heating temperatures were used. For the four higher pre-heating temperatures the milk was passed through two heaters, the first raising the temperature of the milk to about 150° F., the second completing the required heating. For the lowest pre-heating temperature of 160° F. only the first heater was used. There was some unavoidable fluctuation both below and very slightly above 160° F., but the four higher pre-heating temperatures were accurately maintained. Each pre-heating temperature was adopted for two successive days, samples of the powder for storage and other tests being taken on the second day. In this way there was no risk of the sample being contaminated with powder made earlier from milk which had been pre-heated to a different temperature.

PART I. THE BACTERIOLOGICAL TESTS

METHODS

Sampling. Samples of raw, pre-heated and condensed milks and of the milk powder were obtained from two different batches on the second day of each pre-heating temperature. The raw milk was sampled from the top of full tanks of milk using a sterile dipper or from partially full tanks at the joint in the outflow pipe at the bottom of the tank. The pre-heated milk was sampled from a tap, which was well flushed before sampling and which was situated at the end of the horizontal pipeline leading from the pre-heater and about 4 ft. from the entrance to the 'balance tank'. This sample was taken before the application of the vacuum which is maintained from the exit of the pre-heater to the condenser.

The condensed milk was sampled twice, first as it entered the holding tank and again $1\frac{1}{2}$–2 hr. later. Since each batch of condensed milk took approximately 2 hr. to dry, the samples were taken at the beginning and towards the end of the drying period, so that any changes taking place while the condensed milk was being held might be determined.

Milk-powder samples were taken direct from the sifter sleeve towards the end of the drying period and immediately after taking the second sample of condensed milk.

Examination. The milk samples were immediately cooled in brine and examined within 30 min. by plate counts at 37 and 55° C. (3 days' incubation) on yeastrel milk agar and by the presumptive coliform test.

The milk-powder samples were examined 14 days after manufacture. They were reconstituted by the two different techniques A and B which have been described by Higginbottom (4). In technique A, 10 g. powder were added to 90 ml. quarter-strength Ringer solution at room temperature and shaken through a distance of 1 ft. fifty times in 12 sec. Plates were prepared between 5 and 15 min. after reconstitution. In technique B, the Ringer solution was heated to 50° C. before the powder was added to it. The degree of shaking was modified to twenty-five excursions of 1 ft. in 12 sec. and the reconstituted milk was held at 50° C. for 15 min. before plating.

Plate counts for the reconstituted powder were determined on yeastrel milk agar after incubation for 3 days at 37 and 55° C., and for 5 days at 30° C. The spore count (3 days at 37° C.) was determined after heating the reconstituted milk at 80° C. for 10 min. The keeping quality of the reconstituted milk at 60° F. was taken as being the time which had to elapse before the milk gave a clot on boiling.

Results

The results for the liquid milks are shown in Table 1 and for the powders in Table 2.

In presenting the results (Table 1) the counts after 3 days at 30° C. and 5 days at 37° C. have been omitted, since they were so similar to the counts after 5 days at 30° C. and 3 days at 37° C. that they did not affect the interpretation of the results.

The raw milk was of poor bacteriological quality throughout the experimental period with counts of over ten millions per ml. and positive coliform tests in the highest dilution tested, i.e. 1 in 10,000. The thermophile count (55° C.) was variable, and relatively high in half the samples. The use of raw milk with such high counts would be expected to show clearly the relative efficiency of the different pre-heating temperatures in reducing the numbers of viable organisms. This was found to be so. Reference to Table 1 shows that as the pre-heating temperature was lowered from 200 to 160° F. an increasing number of organisms capable of growing at 37 or at 55° C. survived the pre-heating conditions.

With the condensed milk, the pre-heating temperature had a similar effect. The counts at 37° C. for the condensed milk made from milk which had been pre-heated to 200 and 190° F. were very low compared with the corresponding counts where the pre-heating temperatures had been 170 and 160° F. The thermophile counts (55° C.) for the condensed milk samples were variable and did not appear to be related either to the pre-heating temperature or to the thermophile count of the raw milk.

The temperature of the condensed milk in the feed tank supplying the spray-drier varied from about 134 to 139° F. in the 1½–2 hr. during which the condensed milk was being dried. Where the higher pre-heating temperatures had been used there was a tendency for the low initial counts at 37° C. to increase slightly during this holding period especially in the later batches, but where the lower pre-heating temperatures had been used, the relatively higher initial counts tended to decrease.

In six of the condensed milk samples positive coliform tests were obtained at the first sampling, although in all the pre-heated milks the test was negative in 1 ml. 1½–2 hr. later the test for all six samples was again negative. The explanation for this observation is not known. All the positive tests were confirmed on plating out yielding *B. coli* or *coli*-like

Table 1. *The bacterial counts of the raw, pre-heated and condensed milks*
(The incubation period was 3 days throughout)

Table 1. *The bacterial counts of the raw, pre-heated and condensed milks*

(The incubation period was 3 days throughout)

Pre-heating temp. ° F.	Batch no.	Raw milk Plate count/ml. 37° C.	Raw milk Plate count/ml. 55° C.	Pre-heated milk Plate count/ml. 37° C.	Pre-heated milk Plate count/ml. 55° C.	Condensed milk (*a*) 1st sampling Presumptive coliform test ml.	Condensed milk (*a*) 1st sampling Plate count/ml. 37° C.	Condensed milk (*a*) 1st sampling Plate count/ml. 55° C.	Condensed milk (*b*) 2nd sampling, 1½–2 hr. later Plate count/ml. 37° C.	Condensed milk (*b*) 2nd sampling, 1½–2 hr. later Plate count/ml. 55° C.
200	1	29,600,000	107	430	36	+1/10	15,500	2,700	26,400	6,050
	3	25,650,000	15,710	18	62	−1	12,550	7,000	95,500	13,650
190	1	20,550,000	7,990	2,060	32	−1	8,850	5,250	7,850	4,700
	3	20,550,000	19	6,550	16	−1	6,900	45,600	31,500	28,050
180	2	15,600,000	33,800	108,560	1,500	+1	17,950	17,670	17,150	27,150
	4	105,000,000	7,850	31,160	224	+1/10	20,350	329,500	22,150	224,000
170	3	28,550,000	824	166,000	2,510	+1	837,000	9,300	378,500	8,400
	4	20,200,000	8	355,500	33,200	−1	322,500	18,250	210,500	14,350
160	1	154,500,000	33	660,000	120	+1	150,000	4,300	143,000	2,010
	2	15,250,000	11,450	660,000	12,100	+1	581,500	5,800	225,000	4,700

The presumptive coliform test: Raw milk—positive for all samples in 1 in 10,000 (highest dilution tested).
Pre-heated milk—negative in 1 ml. for all samples.
Condensed milk, 2nd sampling—negative in 1 ml. for all samples.

Table 2. *The bacterial count of milk powder made from milk pre-heated at different temperatures and determined 14 days after manufacture*

Pre-heating temp. ° F.	Hours from start of day's drying	Dried milk reconstituted by technique A Plate count/g. powder 3 days at 37° C.	5 days at 30° C.	3 days at 55° C.	Spore count 3 days at 37° C.	Dried milk reconstituted by technique B Plate count/g. powder 3 days at 37° C.	5 days at 30° C.	3 days at 55° C.	Spore count 3 days at 37° C.
200	2	5,300	5,950	70	60	16,250	16,000	700	20
	6	3,650	6,550	150	60	21,600	26,850	320	10
190	2¼	3,200	4,350	100	60	7,950	10,850	120	30
	5¾	3,950	4,700	190	40	21,800	19,000	300	20
180	4	2,100	6,600	180	—	4,500	43,000	820	—
	7	2,600	8,000	1,200	—	12,500	38,000	1,260	—
170	5½	9,650	23,850	500	80	162,000	195,000	370	30
	8	5,300	25,950	780	60	73,500	176,500	840	70
160	2	12,000	218,500	250	50	33,500	605,500	90	20
	4	9,000	54,000	90	60	113,500	314,000	150	10

The presumptive coliform test was negative in 1 ml. reconstituted milk for all samples by both techniques.

types. Only four of the ten coliform organisms isolated survived heating in milk for 30 min. at 145° F.

The effect of the pre-heating temperature upon the bacterial count of the milk powders can readily be seen from the results recorded in Table 2. With the low pre-heating temperatures of 170 and 160° F., the counts at 37° C. were slightly higher and at 30° C. very markedly higher than the corresponding counts for the powders where the three higher pre-heating temperatures were used. The differences were much more clearly brought out when the powders were reconstituted by technique B than they were by technique A and when the incubation temperature was 30 rather than 37° C. For instance, the counts obtained for the pre-heated and condensed milks using 180° F. showed a definite increase in survival of bacteria as compared with the corresponding samples for pre-heating temperatures of 190 and 200° F., but in the powders this difference was only apparent when the powder had been reconstituted at 50° C. and the plates incubated at 30° C. The spore and thermophile counts of the powders were little influenced by the pre-heating temperature. The average time for which the reconstituted milk could be held at 60° F. before a clot was obtained on boiling was 3½ days. Individual samples varied from 3 to 5 days, but the variation bore no relation to pre-heating temperature.

The powders were examined again after storage in gas-tight containers for 6 months at laboratory temperature. The results of this second examination are shown in Table 3. The effect of the pre-heating temperature was still evident although the actual counts were considerably reduced.

Table 3. *The bacterial counts of the milk powder samples after storage for 6 months at room temperature*

		Reconstituted by technique A Plate count			Reconstituted by technique B Plate count		
Pre-heating temp. ° F.	Batch	3 days at 37° C.	5 days at 30° C.	3 days at 55° C.	3 days at 37° C.	5 days at 30° C.	3 days at 55° C.
200	1	1,700	2,200	100	7,800	8,400	1,570
	3	750	650	230	14,300	8,300	470
190	1	2,350	1,900	40	6,600	6,900	80
	3	1,250	900	130	8,650	6,700	290
180	2	1,400	2,250	160	2,450	16,500	320
	4	1,250	3,250	950	5,250	20,600	2,300
170	3	1,600	5,000	290	36,500	58,000	580
	4	750	2,800	290	18,500	51,500	460
160	1	4,000	38,000	40	11,750	220,500	50

Summary to part I

1. With a raw-milk supply of poor bacteriological quality, pre-heating temperatures of 200 and 190° F. greatly reduced the bacterial count in the pre-heated and condensed milks. The reduction in count was less marked when 180° F. was used, and was very considerably less for 170 and 160° F.

2. The bacterial counts of the powders were low for pre-heating temperatures of 200 and 190° F. When 180° F. was used they were only slightly higher, the difference being detected only when the powders were reconstituted at 50° C. and the plates incubated at 30° C. Pre-heating temperatures of 170 and 160° F. resulted in powders of relatively high bacterial counts.

PART II. THE KEEPING PROPERTIES OF THE POWDERS

Each pre-heating temperature was in use for a period of 2 days. On the second day of each period three 21 lb. cans of milk powder were taken for storage tests. Two cans corresponding to each temperature were sent to the Hannah Dairy Research Institute and one can to the Low Temperature Research Station. The storage tests carried out at the two Institutes were similar to those already described in earlier work (1, 5).

PROPERTIES OF THE FRESH POWDERS

Moisture content. The moisture contents of the powders are recorded in Table 4. The values obtained varied according to the method by which they were determined, drying in a vacuum oven at 100° C. giving values which, on the average, were 0·3% higher than those obtained by drying in an air oven at 103° C. and 0·5% higher than those obtained by drying in an air oven at 100° C. Since, however, none of the values exceeded 2% the moisture contents of the five powders stored at the two Institutes were sufficiently low to ensure that the predominating type of deterioration which would be expected to occur in the powders on storage would be oxidation of the fat and not the protein-lactose type of deterioration which predominates in powders of relatively high moisture content.

Table 4. *The properties of the fresh powders*

	Moisture content %			Solubility % at		Copper content p.p.m.		Fat extracted by light petroleum at		
		Cambridge samples								
Pre-heating temp. °F.	Hannah Institute samples Air oven*	Air oven†	Vacuum oven‡	20° C.	50° C.	Hannah Institute samples	Cambridge samples	Room temp. (% of milk powder)	Hot (<40° C.)	Hot (40–60° C.)
200	1·2	1·3	1·7	99	99	1·5	1·3	2·6	4·7	6·3
190	1·6	1·8	2·0	100	99	1·1	1·4	3·2	6·4	9·2
180	1·2	1·5	1·6	100	99	3·6	3·2	2·4	3·3	4·4
170	1·2	1·4	1·7	100	100	3·5	3·3	2·5	3·7	6·0
160	1·3	1·3	1·9	99	99	1·4	1·4	1·4	2·0	2·2
Average	1·3	1·5	1·8	100	99	2·2	2·1	2·4	4·0	5·6

* 3 hr. at 100° C. † 4 hr. at 103° C. ‡ 5 hr. at 100° C. and 3 cm. pressure.

Solubility. The solubility of the powders, as determined by the rapid method of Howat *et al.* (6) making a correction for the hydration of lactose during the determination, was 99 or 100% for all the samples (Table 4). Only a trace of insoluble sediment was visible when 10 ml. of the reconstituted milks were centrifuged, and there was no suggestion of chalkiness in any of the reconstituted samples. The use of high pre-heating temperatures, therefore, had not adversely affected the solubility of the powders.

Copper content. The copper content of the samples was determined by the method of Sylvester & Lampitt (7). The values recorded in Table 4 show that for the powders made from the milk pre-heated to 170 and 180° F., the copper content ranged from 3·2 to 3·6 p.p.m., but that for the other three powders the corresponding values were 1·1–1·5 p.p.m. When converted to a liquid milk basis, the latter values are only very slightly higher than those which have been recorded for normal cows' milk (7). At one stage in the drying plant, the liquid milk had to pass through a tank in which a copper surface was exposed. This was almost certainly the source of the different degrees of copper contamination which occurred on different days.

Readily extractable or 'free' fat. Some of the results of the storage tests in the present work (p. 395) showed that the powders tended to have a longer storage life in lacquered cans than in plain tin-plate cans. It was thought that this might be related to the degree to which the fat in the particles of the powder was 'free' or readily extractable by organic solvents. The amount of 'free' fat in each of the powders was therefore estimated by cold extraction with light petroleum (b.p. 80–100° C.) and also by two different types of boiling petroleum (b.p. < 40° C. and b.p. 40–60° C.). The results in Table 4 show that the hot extraction with light petroleum (b.p. 40–60° C.) extracted rather more fat than the other two methods, and that the five powders contained a somewhat variable proportion of 'free' fat ranging from 2 to 9% of the total powder or from about 7 to 33% of the total fat.

Sulphydryl content. The nitroprusside test for sulphydryl compounds was carried out by a method similar to that described by Josephson & Doan (8) and used by the present authors in the previous work (1), the resulting pink colour being measured in a Lovibond tintometer. The results in Table 5 show that the intensity of the sulphydryl reaction was closely related to the pre-heating temperature.

Table 5. *The sulphydryl test and the volatile sulphur in the fresh powders*

Pre-heating temp. ° F.	Nitroprusside test Lovibond red units*	Method (*a*) lead sulphide stain	Method (*b*) γ volatile sulphur removed from 50 g. milk solids in 1 hr.
200	1·1	Very slight stain in 4 min.;	8·5
190	1·0	dark stain in 30 min.	8·0
180	0·8	No stain in 10 min.; very slight stain in 20 min.; slight stain in 30 min.	1·0
170	0·6	No stain in 60 min.	0·0
160	0·5	No stain in 60 min.	0·0

* 0·5 yellow unit was used throughout.

The presence of volatile sulphur produced from heat-damaged protein was tested for: (*a*) by a modification of the method of Gould & Sommer (9) described in the previous work (1), and (*b*) by a method similar to that described by Townley & Gould (10), which is based on the collection of the volatile sulphur in zinc acetate solution and its colorimetric estimation after conversion to methylene blue.* In method (*a*) nitrogen at the rate of 100 l./hr. was passed through 200 g. milk powder dispersed in 1 l. of water at 60° C., the presence of H_2S being detected by passing the gas through a filter paper disk moistened with lead acetate. In method (*b*) a quantity of the powder containing 50 g. milk solids was reconstituted to give a 12·5% solution and heated to 60° C. under nitrogen in a specially shaped vessel immersed in a thermostat. 60 l. of oxygen-free nitrogen were passed through the solution during 1 hr. and the volatile sulphur absorbed in a small quantity of alkaline zinc acetate solution, using a sintered glass bubbler. The volatile sulphur was estimated colorimetrically after conversion to methylene blue. Data given by both methods are recorded in Table 5. The presence of a higher content of copper which is known to catalyse the oxidative destruction of —SH compounds may possibly have contributed to the greatly weakened reaction of the 180° as compared with the 190° F. sample.

Flavour. The most palatable of the five powders was that made from milk which had been pre-heated to 180° F. It showed no signs of talloviness and possessed a mere trace of

* Lea, C. H. (1946). *Analyst*, **71** (in the press).

boiled or cooked flavour. On the Cambridge scale of assessing flavour, where 6·0 denotes the flavour of fresh liquid milk, this 180° F. powder was awarded a mark of 5·8. The 190 and 200° F. samples had a pronounced boiled or cooked flavour but were nevertheless fresh tasting and palatable. They scored 5·4. The 160 and 170° F. samples had a definite incipient tallowy flavour but were still acceptable as fresh milk powder. They were awarded a mark of 5·0. The three high-temperature milks were therefore regarded as being more palatable than the two low-temperature milks even at the beginning of the storage experiment. At the beginning of the previous experiment with powders made on a Kestner plant(1), a slight preference was expressed for the low-temperature (165° F.) milks as compared with the high-temperature (190° F.) samples, but the low-temperature milks deteriorated more rapidly and were soon inferior to the others.

Storage tests at the Hannah Institute

The contents of the 21 lb. cans of powder corresponding to each pre-heating temperature were packed in 6 oz. Danish cream cans of the 'sanitary, open-top' type at the rate of 90 g./can. The cans were not washed in any way but were used just as they were received from the factory. The volume of this type of can is 186 ml., so that the packing density was 0·484 and, assuming an apparent density of 1·31 for the milk solids(11), the free-oxygen content of the packed cans was 37·3 mg./100 g. powder. The seams of the cans were lacquered externally to minimize the risk of leaks. The cans were stored at 47° C., 37° C. and room temperature. A number of cans were also nitrogen-packed and stored in a refrigerator as controls. To compare the keeping properties of the five powders, gas analysis and tasting tests were carried out at suitable intervals. For the samples at room temperature peroxide values were also determined on a few occasions.

For estimating the peroxide value the method of Chapman & McFarlane(12) in which the fat is extracted with acetone was used, but the moisture content of the powders was first raised to 10 or 12% to facilitate extraction. Values given by this method are probably too high(13), but they provide useful confirmatory evidence on the relative keeping qualities of the stored powders.

For the tasting tests samples of powder were reconstituted at 25° C. in eight times their weight of water, labelled in a code unknown to the tasters, and tasted ½–1 hr. later by five people accustomed to assessing the flavour of milk powder in this way. Each taster wrote a brief description of the flavours observed and the sample was awarded a mark according to the following scheme: 0 = very good and very like ordinary fresh milk, 1 = fairly good and quite palatable, 2 = slightly but definitely unpalatable due to the presence of slight 'off'-flavours, 3 = unpalatable due to the presence of pronounced 'off'-flavours, 4 = very tallowy and most unpalatable. A control powder which had been stored at 0° C. was also included in each test.

The average of the off-flavour marks awarded by the five tasters for each sample at each tasting was plotted against the storage time to give the curves shown in Fig. 1. When the curves so obtained pass an off-flavour score of 1·0, the trained panel is beginning to detect the development of slight off-flavours. When score 2·0 is reached the off-flavour is believed to be sufficiently obvious to be noticed and objected to by the ordinary consumer. The times required for off-flavour marks of 1·0 and 2·0 to be passed have been recorded in Table 6.

The amount of oxygen absorbed by the various powders has been plotted in a similar way (Fig. 2). From the graphs so obtained the times taken for 10 and 20 mg. oxygen to be absorbed per 100 g. powder have been taken for comparing the relative rates at which the powders deteriorated. These times are recorded in Table 7. The relationship between absorption of oxygen and deterioration in flavour is shown in Table 8, the temperature coefficients for deterioration in flavour and for absorption of oxygen in Table 9 and peroxide values in Table 10.

Storage tests at Cambridge

The five powders were packed into 315 ml. (A 1) 'sanitary' ('open-top') type cans, at the rate of 128 g. (4½ oz.) per can, corresponding to a packing density of 0·406 g./ml. and a free-oxygen content of 46·8 mg./100 g. of powder. The cans were made completely gas-tight by the application of bitumen to the seams. Absorption of oxygen and production of carbon dioxide by the powders could then be followed by analysis of the head-space gas in the cans as previously described (14).

Two types of container were used: (*a*) a double lacquered* tin-plate can, as used for canned fruit, and (*b*) a plain tin-plate can. The plain tin-plate cans were washed with peroxide-free ether and hot alcohol before use in order to eliminate the film of grease normally present, which it was thought might influence, possibly in a non-uniform way, the rate of spoilage of the powder.

Constant-temperature rooms at 47, 37 and 15° C. were used for the storage experiments, gas-packed samples being held at −20° C. for use as controls in the tasting tests. For this latter purpose the most palatable (180° F.) powder was employed during the first part of the experiment, but showed signs of a slight deterioration in flavour on prolonged storage† and was replaced by the 190° F. powder for the latter part of the experiment.

For tasting, the contents of a can were mixed and 56 g. reconstituted with distilled water at 20° C. in the ratio of 14 g. powder to 100 ml. water. The samples, labelled in a code unknown to the tasters, were tasted ½–1 hr. later by a panel of six persons, marks being awarded according to the scale 6 = very good, 5 = good, 4 = fairly good, 3 = fair, 2 = rather poor, 1 = poor, 0 = very poor, a system which has been described more fully elsewhere (1).

Peroxide values were determined at intervals on fat extracted by peroxide-free ether from the powder, of which the moisture content had been raised to > 10 %, by both the iodometric method of Lea (15) and the ferric thiocyanate method of Chapman & McFarlane (12).

The tasting results are shown in Fig. 3 and the oxygen absorption results in Fig. 4.

In Table 6 are given the times required for the powders to deteriorate to flavour score 4, which may be regarded as the end of the storage life of the powder as a product of reasonably good quality, and to flavour score 2, at which point the reconstituted milk is becoming definitely unpleasant. The times are given in weeks, and as a multiple of the period required for deterioration of the least stable sample. Table 7 gives similar data for the absorption of 10 and 20 mg. of oxygen per 100 g. of the powders. The relationship between absorption

* Natural, oleo-resinous, acid-resisting type.

† It has now been noticed on several occasions that a powder stored in a restricted supply of oxygen may, after sufficiently long storage, show more deterioration in flavour at −20° C. than at 15° C. The cause of this unusual behaviour which was not observed in the earlier experiments on gas storage (14) may be associated with the observation that smaller quantities of oxygen are required for production of a given degree of off-flavour at low than at high temperatures (Table 8).

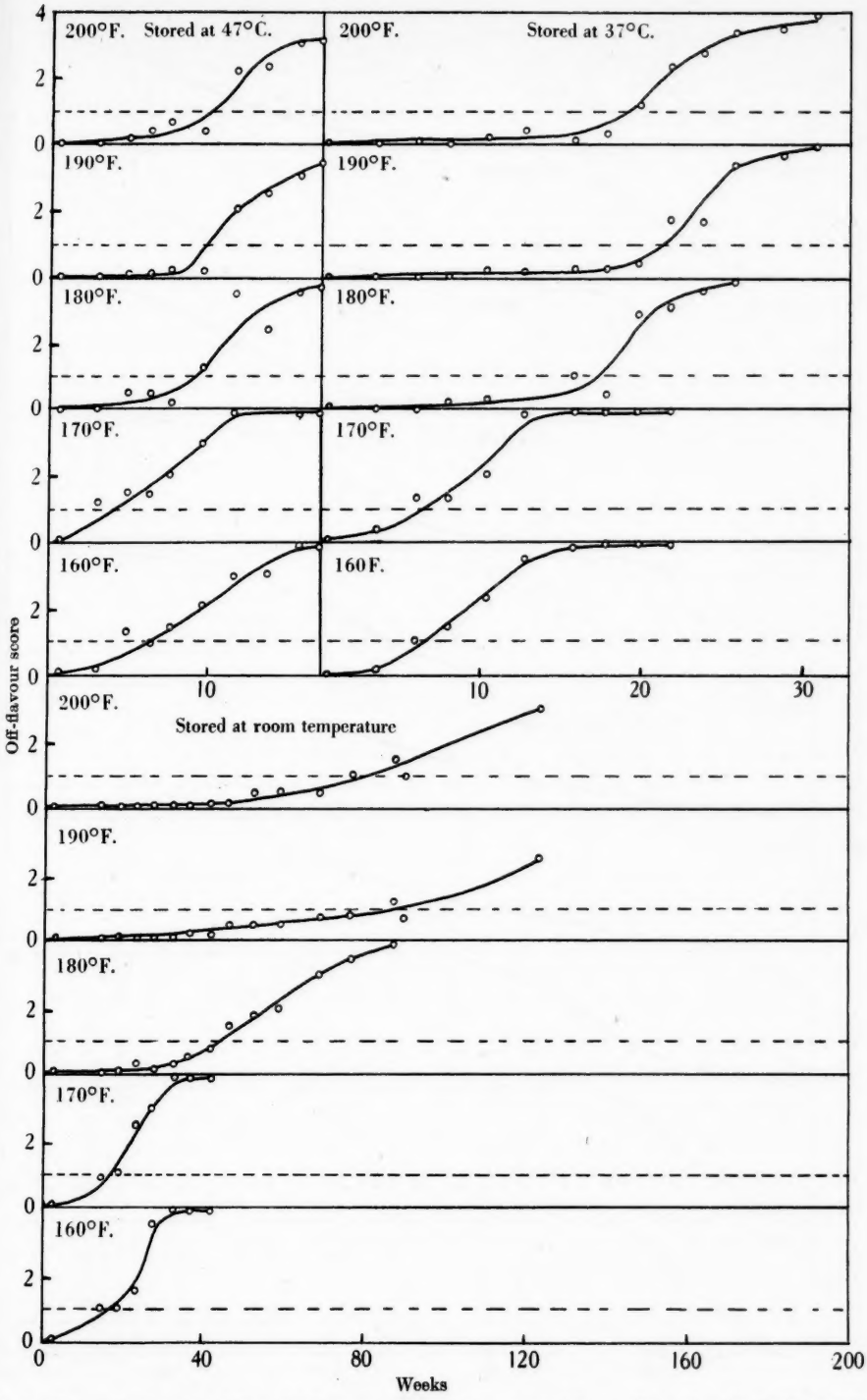

Fig. 1. Changes in the palatability of full-cream milk powders prepared from milk pre-heated at 160–200° F. during storage in plain tin-plate cans at 47°, 37° C. and room temperature (Hannah Institute results).

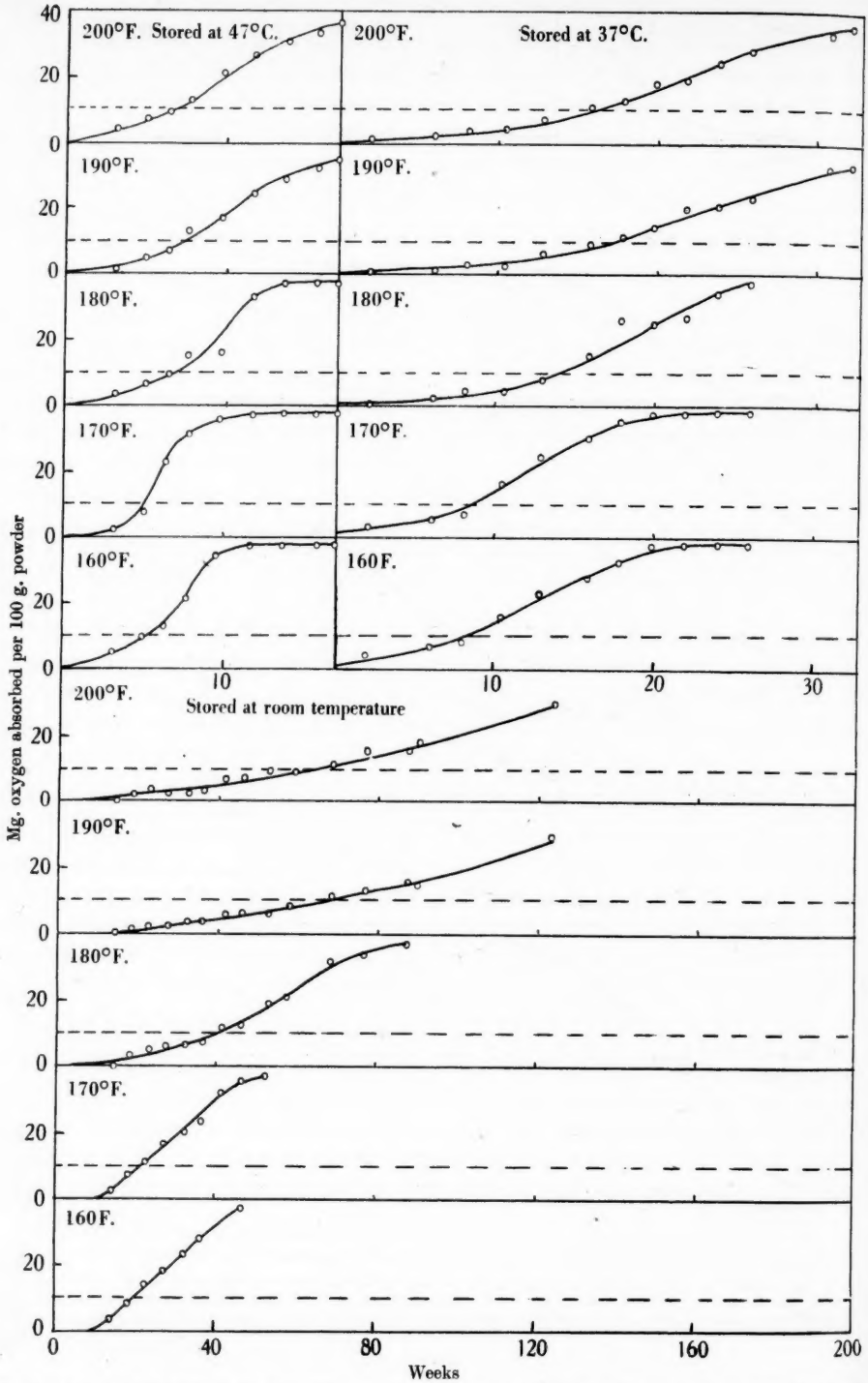

Fig. 2. Absorption of oxygen by full-cream milk powders prepared from milk pre-heated at 160–200° F. during storage in plain tin-plate cans at 47°, 37° C. and room temperature (Hannah Institute results).

Table 6. *The keeping properties of the powders as measured by tasting tests*

Pre-heating temp. °F.	Hannah Institute results (plain cans): Weeks to deteriorate to off-flavour score 1·0			Hannah Institute results (plain cans): Weeks to deteriorate to off-flavour score 2·0			Cambridge results: Weeks to deteriorate to flavour score 4·0 — Lacquered cans			Cambridge results: Weeks to deteriorate to flavour score 4·0 — Plain cans			Cambridge results: Weeks to deteriorate to flavour score 2·0 — Lacquered cans			Cambridge results: Weeks to deteriorate to flavour score 2·0 — Plain cans			Cambridge results: Lacquered plain ratio — At flavour 4·0			Cambridge results: Lacquered plain ratio — At flavour 2·0		
	47° C.	37° C.	Room temp.	47° C.	37° C.	Room temp.	47° C.	37° C.	15° C.	47° C.	37° C.	15° C.	47° C.	37° C.	15° C.	47° C.	37° C.	15° C.	47° C.	37° C.	15° C.	47° C.	37° C.	15° C.
200	10·0	19·2	84	11·8	21·4	102	12·2	26·2	110	6·5	16·0	87	15·0	30·0	>122	8·4	21·0	120	1·9	1·6	1·3	1·8	1·4	—
190	10·2	22·0	88	12·0	23·6	114	11·4	22·4	104	6·5	12·4	89	15·0	27·4	>122	7·6	20·0	127	1·8	1·8	1·2	2·0	1·4	—
180	9·2	17·6	44	11·2	19·6	56	7·2	14·8	48	5·2	10·1	48	9·0	17·4	75	6·7	14·0	71	1·4	1·5	1·0	1·3	1·2	1·1
170	4·1	6·4	16	7·1	9·7	21	3·3	5·8	17	2·8	5·2	17	6·1	9·6	26	4·5	7·6	25	1·2	1·1	1·0	1·4	1·3	1·0
160	5·6	6·0	16	9·6	9·3	24	4·9	7·6	16	3·6	6·0	16	7·9	12·1	25	6·2	11·3	25	1·4	1·3	1·0	1·3	1·1	1·0
	Relative storage life as measured by taste																							
200	2·4	3·2	5·3	1·7	2·3	4·9	3·7	4·6	6·9	2·3	3·1	5·4	2·5	3·1	>4·9	1·9	2·8	4·8						
190	2·5	3·7	5·5	1·7	2·5	5·4	3·5	3·9	6·5	2·3	2·4	5·6	2·5	2·9	>4·9	1·7	2·6	5·1						
180	2·2	2·9	2·7	1·6	2·1	2·7	2·2	2·6	3·0	1·9	2·0	3·0	1·5	1·8	3·0	1·5	1·8	2·8						
170	1·0	1·1	1·0	1·0	1·0	1·0	1·0	1·0	1·1	1·0	1·0	1·1	1·0	1·0	1·0	1·0	1·0	1·0						
160	1·4	1·0	1·0	1·4	1·0	1·1	1·5	1·3	1·0	1·3	1·2	1·0	1·3	1·3	1·0	1·4	1·5	1·0						

Table 7. *The rates of absorption of oxygen by the powders.*

Pre-heating temp. °F.	Hannah Institute results (plain cans): Weeks to absorb 10 mg./100 g. powder			Hannah Institute results (plain cans): Weeks to absorb 20 mg./100 g. powder			Cambridge results: Weeks to absorb 10 mg./100 g. powder — Lacquered cans			Cambridge results: Weeks to absorb 10 mg./100 g. powder — Plain cans			Cambridge results: Weeks to absorb 20 mg./100 g. powder — Lacquered cans			Cambridge results: Weeks to absorb 20 mg./100 g. powder — Plain cans			Cambridge results: Lacquered plain ratio — At 10 mg.			Cambridge results: Lacquered plain ratio — At 20 mg.		
	47° C.	37° C.	Room temp.	47° C.	37° C.	Room temp.	47° C.	37° C.	15° C.	47° C.	37° C.	15° C.	47° C.	37° C.	15° C.	47° C.	37° C.	15° C.	47° C.	37° C.	15° C.	47° C.	37° C.	15° C.
200	6·5	15·6	63	10·0	21·4	97	9·1	21·0	114	5·6	12·1	83	14·5	30·5	>122	8·6	20·0	120	1·6	1·7	1·4	1·7	1·5	—
190	7·4	17·0	64	10·6	23·6	102	8·8	20·0	113	5·1	10·8	92	14·5	27·2	>122	8·0	17·3	130	1·7	1·8	1·2	1·8	1·6	—
180	6·8	14·0	40	9·6	18·6	56	6·2	12·6	59	5·0	10·2	57	8·6	17·7	80	7·5	15·1	78	1·2	1·2	1·0	1·2	1·2	1·0
170	5·2	8·4	21	6·4	11·6	32	4·4	8·0	29	3·8	7·4	29	6·3	11·5	42	5·7	10·9	41	1·2	1·1	1·0	1·1	1·1	1·0
160	5·4	8·0	20	7·6	12·4	30	5·2	8·8	26	4·6	8·1	26	7·5	12·8	39	6·9	12·0	39	1·1	1·1	1·0	1·1	1·1	1·0
	Relative storage life as measured by oxygen absorption																							
200	1·2	1·8	3·2	1·6	1·8	3·2	2·1	2·6	4·4	1·5	1·6	3·2	2·3	2·7	>3·1	1·5	1·8	3·1						
190	1·4	2·1	3·2	1·7	2·0	3·4	2·0	2·5	4·3	1·3	1·5	3·5	2·3	2·4	>3·1	1·4	1·6	3·3						
180	1·3	1·7	2·0	1·5	1·6	1·9	1·4	1·6	2·3	1·3	1·4	2·2	1·4	1·5	2·1	1·3	1·4	2·0						
170	1·0	1·0	1·0	1·0	1·0	1·1	1·0	1·0	1·1	1·0	1·0	1·1	1·0	1·0	1·1	1·0	1·0	1·1						
160	1·0	1·0	1·0	1·2	1·1	1·0	1·2	1·1	1·0	1·2	1·1	1·0	1·2	1·1	1·0	1·2	1·1	1·0						

Table 8. *Relationship between absorption of oxygen and deterioration in flavour*

Storage temp. ° C.	Pre-heating temp. ° F.	Hannah Institute results: mg. oxygen absorbed for deterioration to off-flavour score 1·0	Hannah Institute results: mg. oxygen absorbed for deterioration to off-flavour score 2·0	Hannah Institute results: Difference	Cambridge results: mg. oxygen absorbed for deterioration to flavour score 4·0 — Lacquered cans	Cambridge results: mg. oxygen absorbed for deterioration to flavour score 2·0 — Lacquered cans	Cambridge results: Difference — Lacquered cans	Cambridge results: mg. oxygen absorbed for deterioration to flavour score 4·0 — Plain tin-plate cans	Cambridge results: mg. oxygen absorbed for deterioration to flavour score 2·0 — Plain tin-plate cans	Cambridge results: Difference — Plain tin-plate cans
47	200	20	26	6	15	22	7	13	20	7
	190	19	25	6	14	22	8	12	20	8
	180	18	29	11	13	21	8	11	17	6
	170	5	27	22	8	17	9	6	13	7
	160	11	32	21	9	20	11	7	17	10
	Average			13			9			8
37	200	16	20	4	14	21	7	13	22	9
	190	17	20	3	11	18	7	12	24	12
	180	18	23	5	12	19	7	10	17	7
	170	7	13	6	6	13	7	5	11	6
	160	7	12	5	8	18	10	6	17	11
	Average			5			8			9
15 (Cambridge) or room temperature (Hannah Institute)	200	16	21	5	10	—	—	9	19	10
	190	15	24	9	10	—	—	9	19	10
	180	12	20	8	7	17	10	7	16	9
	170	4	8	4	3	8	5	3	8	5
	160	5	14	9	4	10	6	4	10	6
	Average			7			—			8

Table 9. *Temperature coefficients* for deterioration in flavour and absorption of oxygen*

Pre-heating temp. °F.	Hannah Institute results: 47–37° C. Measured at off-flavour score 1·0	Hannah Institute results: 37–17° C.† Measured at off-flavour score 1·0	Hannah Institute results: 47–37° C. Measured at off-flavour score 2·0	Hannah Institute results: 37–17° C.† Measured at off-flavour score 2·0	Average of 47–37° C. values	Average of 37–17° C.† values
200	1·9	2·1	1·8	2·2	1·9	2·1
190	2·2	2·0	2·0	2·2	2·1	2·1
180	1·9	1·6	1·8	1·7	1·9	1·7
170	1·6	1·6	1·4	1·5	1·5	1·6
160	1·1	1·6	1·0	1·6	1·1	1·6
	Measured at 10 mg. oxygen/100 g. powder		Measured at 20 mg. oxygen/100 g. powder			
200	2·4	2·0	2·1	2·1	2·3	2·0
190	2·3	1·9	2·2	2·1	2·3	2·0
180	2·1	1·7	1·9	1·7	2·0	1·7
170	1·6	1·6	1·8	1·6	1·7	1·6
160	1·5	1·6	1·6	1·6	1·6	1·6

Pre-heating temp. °F.	Cambridge results: Lacquered cans 47–37° C. Measured at flavour score 4·0	Lacquered cans 37–15° C. Measured at flavour score 4·0	Plain cans 47–37° C. Measured at flavour score 4·0	Plain cans 37–15° C. Measured at flavour score 4·0	Lacquered cans 47–37° C. Measured at flavour score 2·0	Lacquered cans 37–15° C. Measured at flavour score 2·0	Plain cans 47–37° C. Measured at flavour score 2·0	Plain cans 37–15° C. Measured at flavour score 2·0	Average of 47–37° C. values	Average of 37–15° C. values
200	2·1	1·9	2·5	2·2	2·0	>1·9	2·5	2·2	2·3	2·1
190	2·0	2·0	1·9	2·4	1·8	>2·0	2·6	2·3	2·1	2·2
180	2·1	1·7	1·9	2·0	1·9	1·9	2·1	2·1	2·0	1·9
170	1·8	1·6	1·7	1·7	1·6	1·6	1·7	1·7	1·7	1·7
160	1·6	1·4	1·7	1·6	1·5	1·4	1·7	1·4	1·6	1·5
	Measured at 10 mg. oxygen/100 g. powder				Measured at 20 mg. oxygen/100 g. powder					
200	2·3	2·2	2·2	2·4	2·1	>1·9	2·3	2·3	2·2	2·3
190	2·3	2·2	2·1	2·6	1·9	>2·0	2·2	2·5	2·1	2·4
180	2·0	2·0	2·0	2·2	2·1	2·0	2·0	2·1	2·0	2·1
170	1·8	1·8	1·9	1·9	1·8	1·8	1·9	1·8	1·8	1·8
160	1·7	1·6	1·8	1·7	1·7	1·7	1·7	1·7	1·7	1·7

* Defined as the ratio of the times required to produce the specified change at two temperatures 10° C. apart.

† It is assumed that the average room temperature approximated to 17° C.

Table 10. *The development of peroxide in the fat of milk powders stored at room temperature and 15° C.*

Hannah Institute results

Table 10. *The development of peroxide in the fat of milk powders stored at room temperature and* 15° C.

Temp. of pre-heating ° F.	Hannah Institute results (acetone extraction) Unwashed tin-plate containers Ferric thiocyanate method Results expressed in m.equiv. peroxide/kg. powder		Cambridge results (ether extraction)									
			(*a*) Iodometric method m.equiv./kg. fat after storage for weeks					(*b*) Ferric thiocyanate method m.equiv./kg. fat after storage for weeks				
	36 weeks	51 weeks	Type of container	48	63	82	102	122	48	63	82	102	122
200	1·8	2·4	Washed tin-plate	3·3	4·9	5·7	7·8	12·6	5·0	9·9	11·8	16·7	25·2
			Lacquered tin-plate	—	2·9	3·5	5·1	7·0	—	5·5	6·5	9·3	11·7
190	2·5	2·0	Washed tin-plate	3·0	2·9	3·5	7·2	10·1	4·5	5·7	8·5	13·2	19·3
			Lacquered tin-plate	—	3·6	4·2	5·2	6·7	—	5·3	7·3	9·0	12·8
180	2·2	10·1	Washed tin-plate	6·7	7·1	15·2	28·2	—	12·7	15·1	25·4	47·6	—
			Lacquered tin-plate	—	8·6	14·7	26·1	—	—	15·9	25·6	43·6	—
170	15·8	16·6	Washed tin-plate	18·4	—	—	—	—	36·8	—	—	—	—
			Lacquered tin-plate	17·3	—	—	—	—	33·7	—	—	—	—
160	12·7	14·2	Washed tin-plate	21·9	—	—	—	—	43·6	—	—	—	—
			Lacquered tin-plate	22·5	—	—	—	—	44·8	—	—	—	—

Table 11. *Correlation between various tests on milk powders stored in plain or lacquered tin-plate containers*

Temp. of pre-heating the liquid milk ° F.	Temp. of storage of the powder ° C.	Correlation between	Hannah Institute results		Cambridge results	
			Degrees of freedom	Correlation coefficient	Degrees of freedom	Correlation coefficient
200, 190, 180, 170, 160	47, 37, 15	Off-flavour score (Hannah Institute), flavour score (Cambridge) and absorption of oxygen	135	0·94	167	−0·86
200, 190, 180	47, 37	,, ,,	55	0·93	74	−0·92
170, 160	47, 37	,, ,,	27	0·84	37	−0·92
200, 190, 180	15	,, ,,	39	0·92	40	−0·96
170, 160	15	,, ,,	8	0·92	10	−0·96
200, 190, 180	15	Flavour score and iodometric peroxide value	—	—	20	−0·94
200, 190, 180	15	Flavour score and ferric thiocyanate peroxide value	—	—	20	−0·96
200, 190, 180, 170, 160	15	Oxygen absorption and iodometric peroxide value	—	—	27	0·99
200, 190, 180, 170, 160	15	Oxygen absorption and ferric thiocyanate value	—	—	27	0·99
200, 190, 180, 170, 160	15	Iodometric and ferric thiocyanate peroxide values	—	—	27	0·99

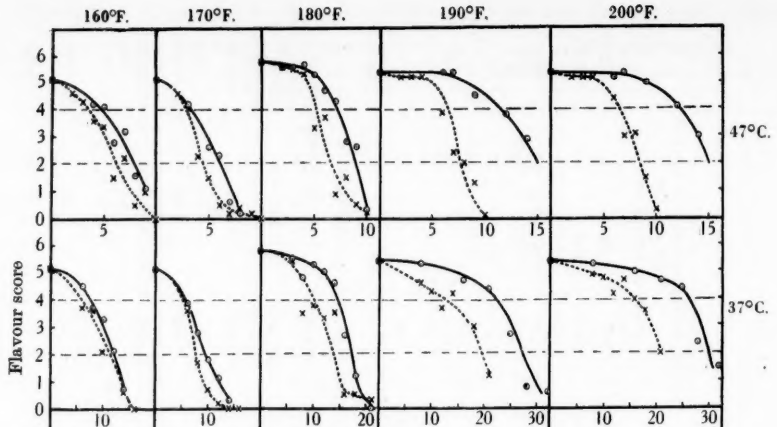

Fig. 3. Changes in the palatability of full-cream milk powders prepared from milk pre-heated at 160–200° F. during storage at 47, 37 and 15° C. (Cambridge results).

⊙ =lacquered. × =washed plain tin-plate can.

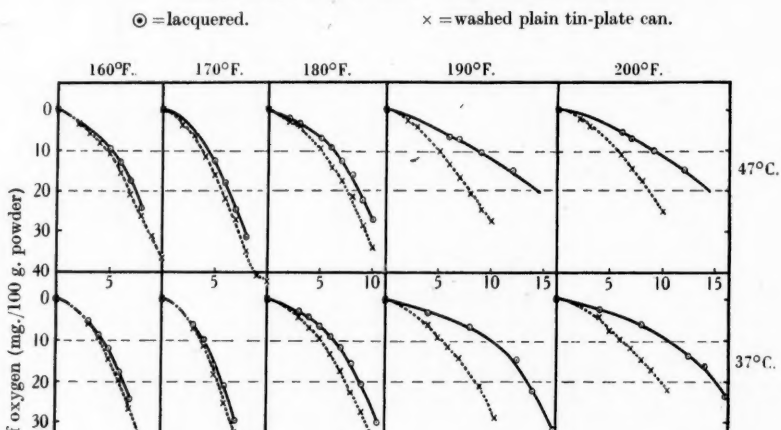

Fig. 4. Absorption of oxygen by full-cream milk powders prepared from milk pre-heated at 160–200° F. during storage at 47, 37 and 15° C. (Cambridge results).

⊙ =lacquered. × =washed plain tin-plate can.

of oxygen and deterioration in flavour is shown in Table 8, the temperature coefficients for deterioration in flavour and for oxygen absorption in Table 9, and peroxide values in Fig. 5 and Table 10.

RESULTS

The effect of the pre-heating temperature on keeping properties

(*a*) *Flavour.* The results in Table 6 show that at 47 and 37° C. the samples made from milk pre-heated to 190 or 200° F. kept in good condition for about two or three times as long as the samples made from milk which had been pre-heated only to 160 or 170° F., when stored in plain tin-plate containers, and about three or four times as long when stored in lacquered tin-plate containers. The corresponding factors for powders from milk pre-heated to 180° F. were somewhat smaller, being about two for both types of container.

For storage at 15° C. or at room temperature the advantage of the higher pre-heating temperatures has been even more marked, the 190 and 200° F. powders keeping for about five times as long and the 180° F. powder for about three times as long as the two low-temperature powders.

Closer inspection of the results shows that the keeping quality of the 170° F. powder did not differ materially from that of the 160° F. powder at the lower storage temperatures, and even tended to be slightly *inferior* at 47 and 37° C. It is quite possible, however, that the relatively high copper content of the 170° F. powder (*c.* 3·4 p.p.m. as compared with 1·4 p.p.m. for the 160° F. powder) may have neutralized any advantage which might otherwise have been gained by raising the pre-heating temperature from 160 to 170° F.

With the 170 and 180° F. powders the copper contents were almost identical, and the increase in pre-heating temperature of 10° F. produced a two- or three-fold increase in storage life.

It is impossible to assess accurately the value of raising the pre-heating temperature a further 10° F. from 180 to 190° F., since this increase was accompanied by a decrease in copper content from *c.* 3·4 to *c.* 1·3 p.p.m. (Table 4), which would itself be expected to cause an extension in storage life. A comparison can, however, be made between the 160 and 190° F. samples, for which the copper contents were practically identical. The great extension in keeping quality obtained by this increase in pre-heating temperature is readily seen from Tables 6 and 7.

Raising the pre-heating temperature from 190 to 200° F. did not appear to cause any very significant difference in the keeping properties of the two powders. On the whole the Hannah Institute results seemed to indicate a slightly longer life for the 190° F. powder. The copper content of the 200° F. powder (1·5 p.p.m.) stored at the Hannah Institute was, however, very slightly higher than that of the corresponding 190° F. powder (1·1 p.p.m.). The copper contents of the Cambridge samples were almost identical (1·3 and 1·4 p.p.m.) and at 47 and 37° C. the 200° F. powder usually appeared to possess a slight advantage in keeping properties (Tables 6 and 7). At 15° C., however, the 200° F. sample was slightly inferior to the 190° F. sample in plain tin-plate and approximately equal to it in lacquered tin-plate, whether deterioration was measured by palatability, oxygen absorption or peroxide value.

(*b*) *Oxygen absorption.* The oxygen absorption results in Table 7 closely confirmed the general conclusions obtained from the tasting results. The rate of deterioration as measured by oxygen absorption was greatest in the 160 and 170° F. powders, and least in the 190 and 200° F. powders, with the 180° F. sample falling between these extremes. Again,

the 170° F. sample appeared at times slightly inferior to the 160° F. sample, but the differences between these powders were always small. Differences between the 190 and 200° F. powders were also small and followed the lines already indicated for taste. For oxygen uptake, as for deterioration in flavour, the advantage of the higher pre-heating temperatures was greater during storage at 15° C. or room temperature than it was at 37 and 47° C. Thus at 37 and 47° C. the higher temperature powders took 1·2–2·6 (average 1·8) times as long to absorb 10 mg. of oxygen as the low-temperature powders, whereas at 15° C. or at room temperature the corresponding factor was about 3·2 (Table 7).

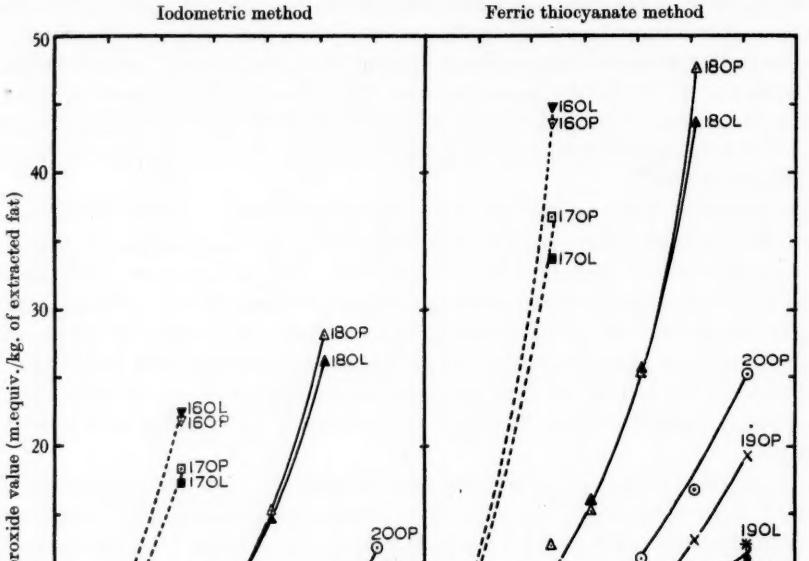

Fig. 5. Development of peroxide in the fat of the various milk powders when stored at 15° C. in washed plain tin-plate and in lacquered tin-plate cans. Initial values for the fresh powders were not determined (Cambridge results).

A comparison of the results in Tables 6 and 7 will show that the advantage of the higher pre-heating temperatures appeared to be greater when judged by taste than when judged by oxygen absorption. For example, at 15° C. or at room temperature the 190 and 200° F. powders kept about five times as long as the 160 and 170° F. powders when judged by taste, but only about three times as long when judged by rate of absorption of oxygen. This fact emphasizes the practical importance of tasting tests in the study of this type of problem, where flavour is the ultimate criterion by which keeping quality is judged.

(*c*) *Peroxide values.* The peroxide values determined for storage at 15° C. and at room temperature (Fig. 5 and Table 10) confirm the principal conclusions obtained from the

tasting tests and show that greatly increased resistance to oxidative changes was possessed by the fat in the two high-temperature powders as compared with the two low-temperature powders, the 180° F. sample falling between the two extremes.

The effect of the container

In Figs. 3 and 4 and in Tables 6 and 7 the rates of deterioration of the powders in lacquered and plain tin-plate containers are directly compared. It can be seen that (*a*) at both 47 and 37° C. there was for every powder a difference in keeping properties in favour of the lacquered container, whether measured by tasting panel score or by absorption of oxygen; (*b*) the difference between the lacquered and plain tin-plate containers was smaller for the unstable 160 and 170° F. powders than for the more stable 190 and 200° F. powders; and (*c*) results at 15° C. indicate that the lacquered/plain difference was much smaller at this temperature than at 47 or 37° C., in fact, it could not be detected for the 160° F. powders and was only very slight for the 170 and 180° F. powders. For the high-temperature milks a definite discrimination in favour of the lacquered container is apparent.

It should be pointed out that there is no doubt about the reality of even the smallest of the lacquered/plain differences observed. For example, the 160 and 170° F. powders in lacquered cans at 47 and 37° C. required on the average only about 1·1 times as long to absorb the specified quantities of oxygen as did the same powders in tin-plate, yet in all of the eighteen pairs of oxygen-absorption figures recorded the lacquered can showed the lower figure of the two. The tasting results were nearly as definite. The powders in the lacquered cans required, on the average, about 1·2–1·3 times as long to deteriorate to a flavour score of 4 or 2 as did the powder in the plain cans, and of the twenty pairs of samples tasted the panel verdict favoured the lacquered can nineteen times and the plain can only once.

Relationship between absorption of oxygen and deterioration in flavour

Comparison of the data on taste and oxygen absorption for the five powders under the various conditions of storage showed that, although a general parallelism existed between oxygen absorption and development of off-flavour, the ratio of the quantity of oxygen absorbed to the degree of off-flavour produced was not constant.

The figures given in Table 8, which have been read off from the smoothed flavour/oxygen absorption curve drawn for each sample and set of conditions, illustrate the magnitude of the variation observed. It can be seen that at 47, 37 and 15° C. or room temperature the least stable (160 or 170° F.) powders required, on the average, about half as much oxygen as did the most stable (190 or 200° F.) powders to deteriorate to a flavour score of 4 or an off-flavour score of 1. On the other hand, the samples usually absorbed roughly the same quantity of oxygen for a further reduction in flavour score from 4 to 2, or increase in off-flavour score from 1 to 2.

Secondary points noted are that (*a*) slightly more oxygen was required at 47 than at 37° C., and considerably more at 37 than at 15° C. or room temperature for the first stage of deterioration in flavour; and (*b*) slightly more oxygen was required by the powder in the lacquered than in the plain tin-plate can for the first stage of deterioration in flavour, except where there was no appreciable difference in keeping properties in the two containers (e.g. 160, 170 and 180° F. powders at 15° C.).

A possible explanation of these observations is considered later (p. 397).

Pearce (16), as the result of an examination of powders from several producers manufactured over a period of 3 months and stored for various periods at temperatures ranging from 0 to 60° C., in air or in inert gas, in light or in darkness (thirty-eight specimens in all), came to the conclusion that none of a large number of chemical and physical tests investigated, with the possible exception of determination of titratable acidity, gave any significant correlation with quality as measured by a tasting panel.

Under the much more restricted conditions employed in the present experiments highly significant correlations between flavour and chemical data, particularly absorption of oxygen, have been observed (Table 11), and the chemical determinations have been found to afford very useful confirmation of conclusions reached by the tasting method.

The susceptibility of the relationships between taste and chemical criteria to very serious disturbance by sufficiently large variations in the conditions of preparation or storage of the powder is, however, well recognized. Variation in moisture or copper content, in temperature of storage or in exposure to light, for example, might even change the type of deterioration responsible for the development of 'off'-flavour from predominantly lipoid to mainly protein, or vice versa.

Temperature coefficients

The relationship between the rates of deterioration at the various temperatures studied is summarized in Table 9 both for deterioration in flavour and for absorption of oxygen. A marked tendency can be seen in the results from both laboratories for the temperature coefficients, whether estimated from deterioration in flavour or from absorption of oxygen, to be lower for the low-temperature powders than for the high-temperature powders. In general, the agreement between the temperature coefficients as determined by taste and those determined by oxygen absorption is very close, except for the Hannah Institute values in the range 47–37° C., where the figures based on oxygen absorption are appreciably higher than those based on flavour.

Discussion

Until some chemical test is devised which will measure deterioration in flavour more accurately than does taste, palatability tests will continue to be the ultimate criterion by which the keeping quality of dried milk is judged. The weaknesses inherent in tasting tests are well known. It is true, for example, that a tasting panel soon settles down to a conventional system of scoring and will produce reasonably consistent and reproducible results, while possibly differing considerably from another panel in its views on such points as the relative degree of undesirability of 'off' or foreign flavours of different types, e.g. incipient tallowy and cooked flavours. It was to minimize such difficulties as well as to overcome other possible weaknesses in technique that the system of duplicating the storage experiments at the Hannah Dairy Research Institute and the Low Temperature Research Station was instituted. In the present work the two laboratories have operated quite independently, each choosing its own technique. Both panels reached a standard of reproducibility adequate for the purpose in view, and a detailed examination of the results of the present work shows that in general the tasting tests at each of the two Institutes were supported by the oxygen absorption and peroxide results, and that the results of one panel were confirmed by those of the other in the main points at issue.

The most important conclusion arising from the joint work is the fact that the milk powder made from milk pre-heated to 190 or 200° F. kept for two to five times as long as powder made from milk pre-heated to 160 or 170° F., the magnitude of the factor depending upon the degree of off-flavour at which it was measured, the temperature of storage and the type of container (Table 6). On storage at room temperature or 15° C. the two highest temperature powders kept for approximately five times as long as the two lowest temperature powders whether they were packed in plain tin-plate used just as it came from the manufacturer (Hannah Institute results) or in plain, grease-free tin-plate or lacquered tin-plate (Cambridge results). The storage life of the 160 and 170° F. powders was about 4 months and that of the 190 and 200° F. powders about 22 months. The 180° F. sample remained palatable under the same conditions for about 11 months, but its storage life may have been somewhat adversely affected as a result of its relatively high copper content.

At the outset the powders prepared from milk pre-heated only to 160 or 170° F. had a slight 'incipient tallowy' flavour when first examined a few days after manufacture and deteriorated rapidly, requiring less oxygen for deterioration to a given flavour score than did milks pre-heated at a higher temperature. The most reasonable interpretation of these observations seems to be that the low-temperature (160 and 170° F.) samples had already, during drying, absorbed an appreciable quantity of oxygen, the effect of which was, in fact, detectable in the lower initial flavour score. Another factor probably involved is absorption of oxygen by the non-fatty constituents of the milk which, during the induction period of fat* oxidation, may account for a considerable proportion of the total absorption. Experience with other food systems and in small degree with milk powder (14) suggests that such changes have a higher temperature coefficient than fat oxidation, and the increase in temperature coefficient observed with increasing temperature of pre-heating (Table 9) is in agreement with this view. If it be assumed that absorption of oxygen by the non-fatty constituents results in a less serious deterioration in flavour than absorption of an equal amount of oxygen by fat, then factors such as pre-heating at a high temperature which tend to lengthen the induction period, and high-storage temperature, should increase the proportion of the oxygen absorbed by the non-fatty constituents of the powder and render necessary a greater total absorption for production of a given degree of off-flavour. At a later stage of deterioration, when fat is oxidizing rapidly, such factors would become less important and the amount of oxygen required for deterioration from flavour score 4 to 2 or off-flavour score 1 to 2 might be expected to be fairly constant, which seems usually to have been true (Table 8). Similar causes are probably also responsible for the observation that with some powders of low moisture content, the advantage of a given inert gas-pack is appreciably greater at high- than at low-storage temperatures.

The figures for volatile sulphur given in Table 2 are consistent with the view that production of —SH-containing compounds is the factor mainly responsible for the increased stability of the high-temperature pasteurized powders and also, incidentally, for their 'boiled' or 'cooked' flavour. The increase in induction period with increasing temperature of pre-heating certainly suggests increased antioxidant activity, just as the shortening of the induction period in the plain tin-plate container suggests a pro-oxidant action of the metallic surface. Experiments with films of butterfat on glass (reported elsewhere†), as well as on the lacquered and plain tin-plates used in this experiment, suggest

* The term 'fat' is intended to cover also phosphatide.

† Lea, C. H. (1946). *J. Soc. chem. Ind., Lond.* **65** (in the press).

that the difference between the lacquered and plain tin-plate was, in fact, due to an acceleration by the latter rather than to a protective action of the former.

The storage tests at 15° C. are not yet complete, but the results so far available show that the accelerating action of a plain tin-plate surface is less at this temperature than at 47 or at 37° C., possibly owing to less efficient contact between the now solid fat and the metallic surface. The harmful effect of a tin-plate container might also be expected to be less serious in a large (21, 56 or 112 lb.) unit than in the small cans used in these experiments. On the other hand, fat oxidation is autocatalytic and produces volatile oxidation products, and an acceleration induced at the surface is liable to spread throughout the mass.

Summary to Part II

1. Storage tests on spray-dried full-cream milk powders prepared from milk pre-heated at 160, 170, 180, 190 and 200° F. for approximately 20 sec. and dried by the Krause process have been carried out at 47, 37 and 15° C. or room temperature. The storage tests were carried out independently at two different research stations. At the Hannah Institute the powders were packed in plain tin-plate containers as received from the factory. At Cambridge grease-free plain tin-plate and lacquered tin-plate containers were used. Deterioration was followed at both stations by a tasting panel and by determination of the amount of oxygen absorbed by the powder and of the accumulation of peroxide in the fat.

2. When fresh the powder pre-heated at 180° F. had the best flavour, followed in order of preference by those pre-heated at 190 and 200° F., which had a definite but quite pleasant 'boiled' or 'cooked' flavour, and by those pre-heated at 160 and 170° F. which had an incipient tallowy flavour, but were nevertheless still acceptable. The 200 and 190° F. powders gave a strong reaction for volatile sulphur, the 180° F. powder a much weaker but quite definite reaction, and the 170 and 160° F. powders a negative reaction. The copper content of the 180° F. powder which, with the 170° F. sample, was the highest of the group, may have been partly responsible for the weakness of the reaction for volatile sulphur given by this sample. The solubility of the 180, 190 and 200° F. powders was not adversely affected by the high pre-heating temperatures, and the moisture contents of all the powders were sufficiently low to prevent any obvious loss of solubility, which remained very good indeed throughout the storage tests.

3. On storage at room temperature or 15° C. the 190 and 200° F. powders kept for about five times, and the 180° F. powder for about three times as long as the 160 and 170° F. powders. At the higher storage temperatures the advantage of the high pre-heating temperatures was still very apparent though slightly less marked.

In some of the tests the 170° F. powder appeared to be inferior to the 160° F. powder and was never very different from it. This may have been due in part to the relatively high copper content of the 170° F. powder, but the very marked difference between the 170 and 180° F. powders, of approximately equal copper content, indicates that the former temperature is likely to be inadequate for stabilization of the powder.

Storage in unwashed tin-plate containers suggested that keeping properties were not improved and might even be slightly impaired by raising the pre-heating temperature from 190 to 200° F. With washed tin-plate and lacquered containers the 200° F. powder usually kept slightly longer than the 190° F. powder at 37 and 47° C., but at 15° C. the

200° F. powder in plain tin-plate was slightly inferior and in lacquered tin-plate approximately equal to the corresponding 190° F. sample.

4. The temperature coefficient for deterioration as measured by flavour change and by oxygen absorption was higher for the high-temperature than for the low-temperature milks. The difference between the keeping properties of the two types of powder was therefore greater at normal than at high storage temperatures.

5. At 47 and 37° C. samples stored in grease-free tin-plate cans deteriorated more rapidly than those in lacquered cans, presumably owing to a catalytic effect of the metallic surface on the rate of oxidation of the fat, or to an accelerated destruction of protective sulphydryl compounds. The average difference in storage life in the two containers was only about 25% for the unstable 160 and 170° F. powders, but rose to about 75% for the more stable 190 and 200° F. powders. Results at 15° C. show that the lacquer-plain difference is much smaller at this than at the higher temperatures, possibly owing to a less effective contact between the now solid fat and the metallic surface of the container.

6. The quantity of oxygen necessary to produce deterioration to flavour score 4 or off-flavour score 1 (considered to mark the end of the storage life of the powder as a product of reasonably good quality) was greater for the 190 and 200° F. than for the 160 and 170° F. powders, probably due in the main to partial oxidation of the fat of the low-temperature samples during drying, but perhaps also to stabilization of the fat in the high-temperature samples which would result in a relatively greater portion of the total oxygen absorption being accounted for by non-fatty constituents of the powder.

The work described above forms part of a joint programme of the Hannah Dairy Research Institute, the Agricultural Research Council and the Food Investigation Board, Department of Scientific and Industrial Research.

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342. A NOTE ON THE DENSITY OF FULL-CREAM MILK SOLIDS

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A knowledge of the density of air-free milk solids is required for calculation of the quantity of oxygen originally enclosed in sealed cans containing milk powder, and for conversion of percentages of oxygen obtained by analysis of the headspace gas to absolute units. Determination of this figure is a matter of some difficulty owing to the presence of entrapped 'air' in the spray-dried product. In the liquid displacement method there is also a danger of error due to incomplete 'wetting' of the particles, or to solution of one or other constituent of the powder, while in the gas-displacement method error may be introduced by solution or adsorption of the gas by the powder. Lea, Moran & Smith (1) obtained values of 1·29 by a pyknometer method with ethylene glycol, and 1·31–1·32 by a nitrogen displacement method for full-cream solids containing 27% of fat. Muers & Anderson (2), on the other hand, reported values of 1·26–1·28 by a liquid-displacement method using propyl alcohol. Further determinations have therefore been made by various modifications of all these methods.

Material

Roller-dried powders were examined as manufactured and after drying *in vacuo* over P_2O_5 at 37° C. or in the vacuum oven at 100° C. Spray-dried powders were reconstituted to 35% solids in water at 40° C. to remove entrapped air and re-dried by freeze-drying to 3–5% moisture content, followed by vacuum drying at 37 or 100° C. as above. Freeze-drying, after homogenization, might be expected to cause minimal damage to the constituents of the milk, though it will not reproduce the precise physical structure of the spray-dried particle. Drying on the steam bath caused obvious damage by caramelization, loss of solubility and aggregation of the fat globules.

Methods

In gas-displacement method A a weighed amount of powder was introduced into a glass bulb and the increase in pressure noted when a known volume of gas was added from a second bulb. In method B a bulb containing the powder was exhausted by means of a two-stage oil pump, and the amount of gas required to refill the bulb to atmospheric pressure and establish equilibrium read off on a gas burette. The apparatus in both cases was used in a constant-temperature room at 20° C.

For the liquid-displacement method ethylene glycol and propyl alcohol were used, air being thoroughly exhausted from between the particles before finally filling up the density bottle. Neither liquid is ideal, since water is soluble in both, lactose is soluble in glycol, and fat is soluble in propyl alcohol.

Results

The results, which are summarized in Table 1, show that for anhydrous-separated milk solids both the gas- and liquid-displacement methods give the same result, but that the value obtained for anhydrous full-cream milk solids is considerably higher by the gas-displacement method.

The influence of fat content and of moisture content on the density of milk solids. Increase

in fat content reduces the density of milk solids (Table 1). Moisture up to 4·7% appeared to have little if any effect on density as measured by the liquid-displacement method. For direct measurement of the apparent density of undried powders by the gas-displacement method it was necessary to correct the readings obtained for the vapour pressure of the powders which, at 20° C., ranged from 1·5 to 4·5 mm. according to type and moisture content. When this correction was made there was again no consistent difference between the densities of undried and anhydrous powders. Presumably the moisture is present in milk powder mainly in combined form and does not behave simply as a diluent.

Table 1. *The apparent densities of milk powders and of anhydrous milk solids at 20° C. as determined by various methods*

			Gas displacement*					Liquid-displacement method	
				Method B					
Powder	Moisture content (%)	Fat content (%)	Method A air	H_2	N_2	air	O_2	Ethylene glycol	Propyl alcohol
Roller separated	4·0	1·0	1·46(5)	1·48	1·48	1·48	1·48	—	1·46(5)
Do. (dried)	—	1·0	1·46(5)	1·46	1·46	1·46(5)	1·46(5)	—	1·46(5)
Roller F.C. no. 1	3·0	26·6	1·31	1·30	1·31(5)	1·32	1·34	—	1·26(5)
Do. (dried)	—	27·4	1·31(5)	1·30	1·31	1·31(5)	1·34	1·31	1·27
Roller F.C. no. 2	2·5	26·4	1·32	1·29	1·30	1·31	1·33	—	1·29
Do. (dried)	—	27·1	1·31	1·30	1·31	1·32	1·34	1·30	1·28
Roller F.C. no. 3 (dried)	—	27·4	—	1·30	1·31	1·32	1·34	—	—
Spray† F.C. no. 1	4·5	25·2	1·30	—	—	—	—	1·30	1·27(5)
Do. (dried)	—	26·4	1·32	—	—	—	—	1·30	1·26(5)
Spray† F.C. no. 2	4·7	26·1	1·30	—	—	—	—	—	1·27
Do. (dried)	—	27·4	1·30	—	—	—	—	1·30	1·26(5)
Spray† F.C. no. 3	4·7	29·7	1·29	—	—	—	—	—	1·26
Do. (dried)	—	31·2	1·29	—	—	—	—	1·29	1·25(5)

* Figures for the undried powders have been corrected for the error introduced into the determination by the vapour pressure of the powder.

† Freed from entrapped air by the method described in the text.

The influence of the nature of the gas used in the gas-displacement method. It has previously been observed that the apparent density of some finely powdered materials increases as the gas used is changed from helium through hydrogen and nitrogen to oxygen, the figure obtained with helium being assumed to be correct and the higher values obtained with the other gases being attributed to adsorption (3, 4). In the present work helium was not available, but values for the three roller-dried full-cream powders in the other gases showed a marked and progressive increase from hydrogen through nitrogen and air to oxygen (Table 1). On the other hand, separated milk powder showed little evidence of any such increase, which suggested that the effect was probably due to solution of gas in the fat rather than to adsorption.

From the averages of the figures quoted in the table the solubilities of hydrogen, nitrogen and oxygen in full-cream milk solids of 27% fat content at 20° C. when the pressure of the gas is 1 atm. can be calculated to be approximately 0·017, 0·023 and 0·040 ml./g. of powder. Figures for the solubility of gases in 'solid' butterfat at 20° C. are not available, nor can they easily be determined. Values recently obtained for liquid butterfat at 40° C., however, show the same gradation from hydrogen through nitrogen to oxygen (5), and calculation to the milk-powder basis gives values of 0·017, 0·028 and 0·045 ml. of gas dissolved per g. of powder. The agreement between the two sets of data

is as close as can be expected in view of the difference in temperature and the change of state of some of the glycerides.

Furthermore, if the observed densities of full-cream milk solids (27% fat) as obtained by displacement of hydrogen, nitrogen, air and oxygen be plotted against the solubilities of the various gases in butterfat at 40° C., and the lines extrapolated, it is found that the density of full-cream milk solids in a gas of zero solubility in butterfat should be approximately 1·27. Calculation from the observed density of 1·465 for separated milk solids of 1% fat content, using a (determined) value of 0·93 for the density of butterfat at 20° C., also gives 1·27 for the density of full-cream solids (27% fat), and this value is probably correct. It is of interest that the liquid-displacement method with propyl alcohol gives values approximating to this figure, in spite of the fact that the liquid recovered after the determination contains a considerable amount of dissolved fat. On the other hand, the figures obtained with glycol, which dissolves lactose, are obviously too high.

Conclusions

The true density of air-free full-cream milk solids of 27% fat content is approximately 1·27. Variation of the fat content within the limits normally found appreciably affects the figure, but moisture up to 4·7% appears to have little or no effect.

Owing to solution of the gases of air in the fat it is necessary, for calculation of the amount of air enclosed in a can of powder, to employ an apparent density of 1·31–1·32, as obtained by air displacement. If allowance is to be made for selective solution of oxygen from the air, as in calculating the amount of oxygen enclosed with milk powder in a sealed container, it will be necessary to use the figure obtained with oxygen, namely, 1·34. Allowance for the space occupied by water vapour (which exerts a partial pressure of 1·5 mm. over a spray-dried full-cream powder of 2% moisture content) reduces the figure to 1·33(5), and this is probably the most accurate value that could be used.

In the foregoing and previous papers(6, 1) a value of 1·31 as obtained by air displacement was assumed for the apparent density of milk solids. Use of 1·33(5) in place of this figure would increase the factor employed for conversion of the oxygen content of the headspace gas to absolute units by about 1% which, at an oxygen absorption of 10 mg./100 g. of powder (= *c.* 5% of the headspace gas) approximates to the limit of accuracy of the gas analysis. Ratios based upon comparison of the times required for absorption of a given amount of oxygen by different samples of powder would be completely unaffected.

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343. STUDIES OF THE BIOLOGICAL VALUE OF THE PROTEINS (NITROGEN) OF DRIED SKIM MILK: VALUE AS AN ADDITION TO BREAD; TESTS WITH YOUNG AND OLDER RATS; EFFECTS OF STORAGE

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A considerable amount of work has been done in recent years on the improvement of the nutritive value of bread by the addition of small amounts of dried skim milk to the flour(1, 2, 3, 4, 5). It is generally agreed that this improvement is largely due to the protein, calcium and riboflavin supplied by the milk. The experiments now to be described deal further with the protein side of the problem.

Our earlier experiments(3) showed that the addition of 2 or 6% milk to white bread resulted in an increase in the biological value of the proteins of the mixture, but no evidence was obtained that, at these levels of feeding, the milk proteins exerted a supplementary effect on those of bread. At the time it was not feasible to make a simultaneous determination on the milk itself. It was suggested that this lack of a supplementary effect was probably due to the relatively low level of the milk additions. At the same time it was pointed out that, during the baking of bread, the proteins in the crust suffer some injury(6, 7), and that it was possible that the milk proteins might be similarly affected by baking to a degree sufficient to inhibit any supplementary effect. To test this point an experiment was planned in which milk was added to bread either before or after baking.

We thought that it might be opportune to inquire at the same time into the effect of age of the rat on the assessment of the biological value of proteins, a problem on which the literature is far from unanimous. French & Mattill(8), for example, have reported biological values for white bread, containing an unstated quantity of milk, of 74 and 83 for young and mature rats respectively. Sumner(9), on the other hand, obtained lower values for dried milk and dried whole egg with 12-months old rats than with rats 40–50 days old.

We decided to carry out the present experiment according to our normal procedure with young actively growing rats and to repeat it with the same animals when active growth had ceased. As a result of the findings of this experiment certain other problems arose, and the results of tests to solve some of them are also included in this report. The term 'biological value of the protein' recurs frequently throughout this paper and to economize space the abbreviation b.v. will be used.

Experimental

National wheatmeal bread made from 85% extraction flour was used in the experiments described below. In all experiments the diets were made to contain about 8% protein; the b.v. and true digestibility of the proteins were determined on rats by the method of Mitchell & Carman(10, 11). In each experiment three litters of four female rats were used; they were 24–29 days old and weighed 49–68 g. at the beginning of the tests. The cages

used and the general technique of the experiments were as described in detail by Henry, Kon & Watson (12). Diets were fed mixed with distilled water, and the animals received each diet in turn in the course of the experiment.

Exp. 1. *Experiments with young and older rats on the effect of baking on the b.v. of the proteins of bread-milk mixtures*

Two types of bread were made, a national wheatmeal loaf and a milk-wheatmeal loaf in which roller-dried skim milk supplied about 20% of the total protein in the bread; this higher level was chosen as it was felt that it would allow a supplementary effect to become evident (3). Actually the milk supplied 23% of the total nitrogen.

The breads were dried and ground as described by Henry, Houston, Kon, Powell, Carter & Halton (3). The two breads and the milk were then analysed for nitrogen and the national wheatmeal bread and the milk were mixed in the correct proportions to produce a mixture having the same nitrogen content as the milk-wheatmeal loaf. Four experimental diets were then made in which about 8% protein was supplied by national wheatmeal bread, roller-dried skim milk, milk-wheatmeal bread and wheatmeal bread + milk. Table 1 gives the composition and analysis of the experimental diets. The dried skim milk was from the same consignment as that used in our earlier experiments with white bread (Henry *et al.* (3)). At the conclusion of the metabolic tests the young animals were placed on our stock diet (13) and the experiment was repeated when they were 4½ months old, i.e. when growth had slowed down considerably, the animals had reached maturity, and weighed some 200–233 g.

Results

The results are given in Table 2. The most striking, if unexpected, result is the b.v. of 55·2 obtained for the roller-dried milk with young rats; this value is considerably lower than the figure of 68 given by Fairbanks & Mitchell (14) for deliberately scorched roller-dried milk. Their determination was carried out at a 10% level of protein intake which, if anything, would tend to yield lower values than an examination at the 8% level used by us. Our sample of milk had been supplied as a typical commercial sample. Chemical analysis showed that it was abnormal and that this was due to excessive addition of alkali prior to drying and to overheating during the drying process.

The value of 48·9 obtained for the national wheatmeal bread is little different from values for white bread of 44·7, 47·4 and 52·2 previously obtained in this laboratory (3, 15). As reported below, a higher figure of 55·8 has since been obtained by us for national wheatmeal bread bought locally.

From b.v. of the proteins of the national wheatmeal bread and of the milk which were obtained with individual rats, a b.v. was calculated for the mixture of proteins present in the wheatmeal-milk bread. Values so obtained were compared statistically by the paired *t*-test (16, 17) with observed values for the wheatmeal-milk bread (53·5) and for the mixture of wheatmeal bread and of milk (52·8). The comparison showed that the calculated mean value (50·3) was significantly lower than the observed values, the differences and their standard errors of the mean being respectively $3{\cdot}2 \pm 0{\cdot}71$ and $2{\cdot}5 \pm 0{\cdot}79$ percentage units, with odds against the differences being due to chance of 1000 : 1 and 111 : 1 at $P = 0{\cdot}95$. It can therefore be accepted that with this particular sample of milk a small but unmistakable supplementary effect was evident.

Table 1. *Composition and analysis of the diets used in the various experiments*

Component	Exp. 1				Exp. 2				Exp. 3				Exp. 4			
	Wheat-meal bread Diet 182	Milk Diet 183	Milk-wheat-meal bread Diet 184	Wheat-meal bread + milk Diet 185	Wheat-meal bread Diet 224	Milk Diet 225	Milk-wheat-meal bread Diet 226	Wheat-meal bread + milk Diet 227	Wheat-meal bread Diet 204	Milk Diet 205	Bread + 2% milk Diet 206	Bread + 6% milk Diet 207	'House-hold' milk Diet 200	Good roller-dried milk Diet 201	Over-neutra-lized milk Diet 202	Over-heated milk Diet 203
Salts (de Loureiro (26))	4·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00
Margarine fat	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00
Potato starch	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00	10·00
Rice starch	27·10	39·77	32·58	32·60	27·51	41·17	31·76	31·76	21·51	40·66	22·85	25·38	42·34	42·64	41·75	42·80
Sugar (granulated, ground)	—	12·00	—	—	—	12·00	—	—	—	12·00	—	—	12·00	12·00	12·00	12·00
National wheatmeal bread	48·90	—	—	37·51	48·49	—	—	40·11	54·49	—	52·10	47·58	—	—	—	—
Milk-wheatmeal bread	—	—	43·42	—	—	—	44·24	—	—	—	—	—	—	—	—	—
Roller-dried* skim milk	—	24·23	—	5·89	—	—	—	—	—	—	—	—	—	—	—	—
Spray-dried† skim milk	—	—	—	—	—	22·83	—	4·13	—	23·34	1·05	3·04	—	—	—	—
'Household' milk	—	—	—	—	—	—	—	—	—	—	—	—	21·66	—	—	—
Good roller-dried milk	—	—	—	—	—	—	—	—	—	—	—	—	—	21·36	—	—
Over-neutralized roller-dried skim milk	—	—	—	—	—	—	—	—	—	—	—	—	—	—	22·25	—
Overheated roller-dried skim milk	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	21·20
% N in food tested	2·618	5·047	2·948	—	2·640	5·353	2·893	—	2·349	5·237	—	—	5·640	5·722	5·491	5·761
% N in diets:																
Young rat exp.	1·217	1·273	1·347	1·312	1·330	1·317	1·330	1·351	1·302	1·287	1·307	1·309	1·298	1·265	1·277	1·259
Mature rat exp.	1·269	1·287	1·252	1·265	1·341	1·324	1·354	1·351	—	—	—	—	—	—	—	—
% Moisture in diets:																
Young rat exp.	9·53	10·63	9·14	9·62	8·62	9·62	9·21	8·62	8·57	10·26	8·62	9·11	9·67	9·37	9·95	9·56
Mature rat exp.	8·98	9·67	9·84	9·81	8·05	8·20	7·69	6·93	—	—	—	—	—	—	—	—

* Abnormal sample, cf. pp. 404 and 408. † Cf. p. 409.

Table 2. *Exp.* 1. (1) *Mean biological values and true digestibilities of various foods, obtained with young rats* (*a*) *and with the same rats when mature* (*b*). (2) *Analysis of variance of the biological values*

(1)

Period	Biological value: Wheatmeal bread Diet 182	Milk Diet 183	Milk-wheatmeal bread Diet 184	Wheatmeal bread + milk Diet 185	True digestibility: Wheatmeal bread Diet 182	Milk Diet 183	Milk-wheatmeal bread Diet 184	Wheatmeal bread + milk Diet 185
				(*a*) Rats when young				
1	49·0	57·7	55·7	52·6	94·7	85·9	91·7	89·5
2	47·4	55·8	52·2	54·1	92·3	86·4	88·1	90·2
3	49·6	55·1	53·4	50·9	95·1	83·9	88·0	89·7
4	49·4	52·2	52·6	53·6	92·3	84·8	87·5	91·1
Mean	48·9	55·2	53·5	52·8	93·6	85·2	88·8	90·1
Range	45·0–54·1	48·5–61·3	48·9–57·8	49·8–55·7	87·8–98·7	78·1–90·3	85·4–94·8	84·8–94·6
				(*b*) Rats when mature				
1	54·8	60·4	56·0*	59·2	90·4	86·1	88·4*	87·3
2	52·0	56·2	54·0	52·7	89·6	88·4	86·6	89·8
3	55·9	56·0	52·1	54·7	90·6	86·1	87·3	89·0
4	60·4	55·0	55·0	56·9	91·4	90·2	95·5	88·1
Mean	55·8	56·9	54·3	55·9	90·5	87·7	89·5	88·6
Range	50·9–64·9	47·7–63·0	41·5–58·9	48·8–61·1	82·2–98·1	81·5–92·3	85·1–98·8	84·5–93·1

* The faecal analysis for one rat was lost in this period. The figure for true digestibility is for two rats only. For the b.v., in order to complete the analysis of variance, the missing value was estimated from the formula of Yates(27):

$$x = \frac{2T_R + 2T_p + 2T_D - T}{3},$$

where T = total of remaining values in square.

T_R = total of remaining values in square for rat in question.

T_p = total of remaining values in square for period in question.

T_D = total of remaining values in square for diet in question.

Where 'square' is replicated (e.g. three litters), totals refer to litter containing x. If there are three litters and two ages, totals are within the litter and age group containing x.

(2)

Component	D.F.	Sum of squares	Mean square	Variance ratio
(*a*) Total	94	1745·92	—	—
(*b*) Litters	2	111·74	55·87	*b*/*m* 3·97 N.S.
(*c*) Age	1	233·44	233·44	*c*/*n* 25·29**, *c*/*k* 14·48**
(*d*) Diets	3	170·00	56·67	*d*/*o* 8·63***, *d*/*g* 3·76 N.S.
(*e*) Periods	3	96·44	32·15	*e*/*o* 4·90**, *e*/*h* 2·30 N.S.
(*f*) Litters × age	2	40·75	20·38	*f*/*o* 3·11 N.S., *b*/*n* 2·21 N.S.
(*g*) Litters × diets	6	90·46	15·08	*g*/*o* 2·29 N.S.
(*h*) Litters × periods	6	84·04	14·01	*h*/*o* 2·14 N.S.
(*i*) Age × diets	3	130·72	43·57	*i*/*o* 6·64**, *i*/*n* 4·72 N.S.
(*j*) Age × periods	3	42·53	14·18	*j*/*o* 2·16 N.S., *j*/*m* 1·01 N.S.
(*k*) Rats within litters	9	145·12	16·12	*k*/*l* 2·46 N.S.
(*l*) Rats within litters × age	9	231·49	25·72	*l*/*o* 3·92**
(*m*) Periods × litters × age	6	84·36	14·06	*m*/*o* 2·14 N.S.
(*n*) Diets × litters × age	6	55·35	9·23	*n*/*o* 1·41 N.S.
(*o*) Error	35	229·48	6·56	—

Significant difference at: 5% = ±2·12 or ±2·24 for the group containing the missing value;
1% = ±2·85 or ±3·01 for the group containing the missing value;
0·1% = ±3·76 or ±3·97 for the group containing the missing value.

Conclusions: Young rats; diets 183, 184 and 185 > 182***, other differences not significant;
Mature rats; diet 183 > 184*, other differences not significant;
Young *v.* mature rats; mature > young with diets 182*** and 185**, differences for diets 183 and 184 not significant.

* Significant at 5%. ** Significant at 1%. *** Significant at 0·1%. N.S. = not significant.

As far as the effects of baking are concerned no difference was observed between the b.v. of the breads to which milk was added before or after the process. This may have been due to the poor quality of the milk which had already been overheated.

When the rats were mature, very similar values (54·3–56·9) were obtained for all foods tested, and Table 2 shows that they were statistically indistinguishable except for the difference between the milk and the milk-wheatmeal loaf. No supplementary relationship was found here between the bread and the milk proteins. For all substances tested higher b.v. were obtained when the rats were mature than when they were rapidly growing,

Table 3. *Exp.* 2. (1) *Mean biological values and true digestibilities of various foods, obtained with young rats* (*a*) *and with the same rats when mature* (*b*). (2) *Analysis of variance of the biological values*

(1)

	Biological value				True digestibility			
Period	Wheatmeal bread Diet 224	Milk Diet 225	Milk-wheatmeal bread Diet 226	Wheatmeal bread + milk Diet 227	Wheatmeal bread Diet 224	Milk Diet 225	Milk-wheatmeal bread Diet 226	Wheatmeal bread + milk Diet 227
				(*a*) Rats when young				
1	56·6	72·4	54·2	56·7	92·8	87·6	86·7	88·9
2	54·1	70·8	63·3	62·1	92·7	89·2	86·8	89·5
3	53·4	69·8	57·3	57·2	91·9	88·1	93·0	90·3
4	55·8	71·4	55·5	59·0	92·3	87·5	87·6	91·7
Mean	55·0	71·1	57·8	58·7	92·4	88·1	88·5	90·1
Range	52·0–59·8	67·3–75·2	52·0–75·7	51·2–64·3	90·8–94·4	83·4–91·6	83·3–95·2	86·7–94·1
				(*b*) Rats when mature				
1	62·0	70·1	71·4	73·6	93·5	87·9	87·9	88·6
2	71·4	74·2	73·3	72·1	86·8	84·0	88·3	89·4
3	62·7	74·9	64·5	64·8	88·5	88·8	85·3	85·9
4	62·4	71·8	68·2	66·2	88·1	86·6	89·7	90·8
Mean	64·6	72·8	69·3	69·2	89·2	86·8	87·8	88·7
Range	47·8–75·8	62·6–79·2	58·3–79·0	60·8–82·9	82·2–95·5	80·8–91·8	82·5–93·7	83·2–96·7

(2)

Component	D.F.	Sum of squares	Mean square		Variance ratio
(*a*) Total	95	6422·75	—		—
(*b*) Litters	2	12·17	6·08	*b*/*m*	1·71 N.S.
(*c*) Age	1	1684·21	1684·21	*c*/*n*	79·8***, *c*/*k* 52·79***
(*d*) Diets	3	1885·15	628·38	*d*/*o*	29·33***, *d*/*g* 39·7***
(*e*) Periods	3	293·14	97·71	*e*/*o*	4·56**, *e*/*h* 6·99*
(*f*) Litters × age	2	170·13	85·06	*f*/*o*	3·97 N.S.
(*g*) Litters × diets	6	94·88	15·81	*g*/*o*	1·36 N.S.
(*h*) Litters × periods	6	83·87	13·98	*h*/*o*	1·53 N.S.
(*i*) Age × diets	3	375·17	125·06	*i*/*o*	5·84**, *i*/*n* 5·96*
(*j*) Age × periods	3	46·97	15·66	*j*/*o*	1·37 N.S., *j*/*m* 1·50 N.S.
(*k*) Rats within litters	9	321·17	35·69	*k*/*l*	1·54 N.S.
(*l*) Rats within litters × age	9	496·02	55·11	*l*/*o*	2·57*
(*m*) Periods × litters × age	6	62·41	10·40	*m*/*o*	2·06 N.S.
(*n*) Diets × litters × age	6	126·03	21·00	*n*/*o*	1·02 N.S.
(*o*) Error	36	771·43	21·43		—

Significant difference at 5% = ±3·84, 1% = ±5·14, 0·1% = ±6·78.

Conclusions: Young rats; diets 224, 226 and 227 < 225***, other differences not significant;
Mature rats; diet 225 > 224***, diets 226 and 227 > 224*, other differences not significant;
Young *v.* mature rats. Diets 224***, 226*** and 227*** mature > young, diet 225 not significant.

* Significant at 5%. ** Significant at 1%. *** Significant at 0·1%. N.S. = not significant.

but only for national wheatmeal bread and for the wheatmeal bread-milk mixture were the differences significant statistically.

The results confirm the findings of French & Mattill (8) and of Osborne & Mendel (18) that bread proteins are more adequate for the maintenance than for the active growth of rats. For milk Sumner (9) found a decrease in the b.v. of proteins from 84 for young rats to 64 for mature animals when the diet contained 8% protein. On the other hand, Chick, Boas-Fixsen, Hutchinson & Jackson (19), feeding milk at a 7% level of protein intake, obtained a b.v. of 86 for adult rats. This value compares favourably with those found in various laboratories with young rats, for milk. Our present results neither confirm nor contradict either of these findings, but the interpretation of the results is complicated by the fact that the milk used by us had such an exceptionally low b.v.

Little need be said about the true digestibilities of the proteins studied. Values of 93·6 and 85·2 were obtained for bread and milk respectively using the young rats; for the mature animals the corresponding figures were 90·5 and 87·7, the bread-milk combinations occupying intermediate positions at both ages. These results are in agreement with those of Sumner (9), who found that the digestibility coefficient of milk was unaffected by the age of the animal used.

The present experiment disclosed that the sample of milk used in our earlier study (3) was abnormal on account of its very low b.v. This milk was originally supplied by a well-known manufacturer as 'best skim powder'. As the experimental rats were ready for the tests the powder was sent direct from the manufacturers to the Research Association of British Flour-Millers at St Albans (now Ministry of Food, Cereals Research Station) for incorporation into the bread, there being no time for a preliminary examination. Subsequently, chemical tests were carried out at the Hannah Dairy Research Institute and at this Institute. These revealed that the powder was of low grade, clearly made from milk over-neutralized with lime. It had a *p*H of 8 and contained 9·1% ash of unusually high alkalinity. Moreover, it showed evidence of overheating as exemplified by dark colour both of the powder and the reconstituted product. The latter had an unusual taste and odour and was mealy and porridgy in consistency. These findings were communicated to the manufacturers who, nevertheless, maintained that the milk was 'normal' according to their standards.

When the very low b.v. of this sample came to light it was decided to repeat that part of our earlier experiments (3) dealing with the protein contribution of the milk. It will be recalled that the vitamin B_1 and riboflavin contents of the sample were within normal limits. The calcium content was high, most probably because of the addition of lime in over-neutralizing, but 95% of it was retained by the animals which received the bread, and there is no doubt that the availability of the milk calcium had not been affected by the abnormal treatment. For these reasons it was not thought necessary to repeat the experiments dealing with the contribution of the milk to the calcium and vitamin content of the bread or with the effect of the addition on its total nutritive value.

We felt it essential, however, to repeat also the work just described on the utilization of protein by rats at different ages and also to inquire in general into the b.v. of commercial milk samples as the sample just tested was clearly not representative. The results of all these tests are described below, but are not reported in the order in which they were carried out.

Exp. 2. *Repetition of Exp.* 1

This experiment was a repetition of the one described on p. 404 with different samples of bread and of milk. The composition and analysis of the diets are given in Table 1. This time the milk supplied 17% of the nitrogen in the bread-milk mixtures and the rats were 5½ months old and weighed 206–264 g. when the second tests were done.

Results

The results are given in Table 3. The b.v. of the sample of milk now examined, 71, though much higher than that of the abnormal sample used in the original study was nevertheless low for good quality powder. This sample was first assayed in 1941 and was from a bulk purchased towards the end of 1939. Successive tests, summarized in Table 4,

Table 4. *Changes on storage of the biological value of a sample of dried skim milk manufactured in September* 1939

Date of test	Biological value	True digestibility
April 1941	88·5	89·8
January 1943	81·3	87·6
March 1943	80·3	96·9
October 1943	75·0	88·1
March 1944	71·1	88·1

disclosed a progressive deterioration in the b.v. of this milk. Similar findings with the proteins of other foods have been reported by Jones, Divine & Gersdorff(20) and quite recently by Mitchell(21). Further work on this aspect of the problem is at present in progress. We realize now that it would have been better if a sample of freshly prepared milk had been used for this repeat study. The results are nevertheless given, but they will have to be interpreted with caution.

With this reservation in mind it will be seen that the results now obtained confirm those of the earlier experiment. Again the baking of bread had no deleterious effect on the proteins of the milk added to the flour. Similarly the b.v. of the proteins of national wheatmeal bread proved higher for the mature animals (value of 64·6) than for the same animals at the stage of rapid growth (value of 55·0) but, as before, the age of the rat made no difference to the estimate of the b.v. of milk, which for old and young rats was 72·8 and 71·1 respectively. This time higher values than with young animals were obtained with the older rats not only for the unsupplemented bread but also for the milk-bread combinations, the values for the latter being 69·3 and 57·8 and 69·2 and 58·7 for old and young rats respectively. No supplementary relationships between the proteins of bread and milk were now observed. The calculated additive value for the young rats was 57·8 and the values actually found were 57·8 and 58·7. For the older rats the differences were of the same order as for the young rats in Exp. 1 but, owing to individual variations, the differences between the calculated (66·0) and observed (69·3 and 69·2) values were without statistical significance. The differences with their standard errors of the mean were $3{\cdot}3 \pm 2{\cdot}34$ and $3{\cdot}2 \pm 1{\cdot}95$ and the paired *t*-test(16, 17) shows that odds against the differences being due to chance were only 5 : 1 and 7 : 1 respectively.

It should be remembered that, in this experiment, milk provided only 17% of the total nitrogen as against 23% in the original study, and this might possibly explain the lack of supplementary relationship now observed, as the critical ratio for mixtures of milk with cereals would seem to be somewhere about 20%(3). It should be noted, however, that the

milk previously used had a very low b.v. and therefore a correspondingly high 'supplementary fraction'(22), and that it was therefore more likely than the sample now used to exhibit supplementary effects.

Exp. 3. *The biological values of the proteins of breads baked from national wheatmeal flour supplemented with* 2 *or* 6% *of dried skim milk*

Our earlier findings with white bread containing 2 and 6% of dried skim milk(3) have already been mentioned on p. 403. We did not assay at the time the b.v. of the proteins of the sample of milk added to the bread, and in calculating the additive value of the protein mixtures we used a figure for milk taken from the literature. These calculations gave no indication of the existence of supplementary relationships between the proteins of white flour and of milk when present in the proportions found in our breads. Twenty-one months later we measured the b.v. of the proteins of that sample of milk and obtained the extremely low value of 55·2 (cf. p. 404). When this value is used for the calculation, figures of 45·2 and 46·1 are obtained for the additive b.v. of the breads containing 2 and 6% milk respectively, while the values found were 47·6 and 49·7.* It seems likely that the b.v. of this milk may have deteriorated between the two experiments. Values quoted in Table 6 indicate that the b.v. of another sample of milk decreased by about half a percentage unit per month. Allowing for the same rate of loss, it can be calculated that the sample under discussion may have had a b.v. of 65·7 at the time when it was added to bread. Additive b.v. calculated on this basis for the 2 and 6% milk breads work out at 45·7 and 47·6 respectively. Even these values are slightly lower than those actually found, and it might be argued that in our earlier experiment some slight supplementation had taken place between the bread and milk proteins.

In the repeat experiments now to be described, national wheatmeal flour of 85% extraction was used instead of the white flour as the latter was no longer available to the public; the milk was only added to the bread after baking. The following foods were examined: national wheatmeal bread, spray-dried skim milk, a mixture of wheatmeal bread and 2% milk, and a similar mixture with 6% milk. The composition and analysis of the diets are given in Table 1 and the results in Table 5.

Results

The b.v. of the milk was 81·3. It should be noted that it was with this milk that the steady decline in the b.v. was noticed as reported in Table 4. The value first observed for this milk(15) was 88·5, but the value now found was still within the normal limits. For

Table 5. *Exp.* 3. (1) *Mean biological values and true digestibilities of various foods.* (2) *Analysis of variance of the biological values*

(1)

	Biological value				True digestibility			
Period	Wheatmeal bread Diet 204	Milk Diet 205	Bread + 2% milk Diet 206	Bread + 6% milk Diet 207	Wheatmeal bread Diet 204	Milk Diet 205	Bread + 2% milk Diet 206	Bread + 6% milk Diet 207
1	51·8	80·1	54·7	59·4	91·2	86·9	92·5	92·5
2	57·6	82·5	55·6	61·7	91·4	88·3	90·5	90·5
3	57·6	81·4	59·5	61·4	90·7	84·6	92·1	90·6
4	56·3	81·3	58·1	61·7	91·0	90·4	90·2	91·0
Mean	55·8	81·3	57·0	61·0	91·5	87·6	91·3	91·2
Range	51·3–60·0	78·7–84·6	53·5–60·9	58·1–64·6	88·4–93·6	72·2–95·3	89·5–93·5	88·6–93·1

* In the original publication ((3), p. 194, lines 9 and 10 from the bottom) the calculated values were given instead of those found and vice versa.

(2)

Component	D.F.	Sum of squares	Mean square		Variance ratio
(*a*) Total	47	5346·94	—		—
(*b*) Litters	2	3·72	1·86	*b*/*h*	1·67 N.S., *b*/*f* 3·12 N.S.
(*c*) Diets	3	5102·81	1700·94	*c*/*h*	225·8***, *c*/*e* 209·6***
(*d*) Periods	3	87·33	29·11	*d*/*h*	9·38***, *d*/*f* 5·02*
(*e*) Litters × diets	6	20·05	3·34	*e*/*h*	1·08 N.S.
(*f*) Litters × periods	6	34·82	5·80	*f*/*h*	1·87 N.S.
(*g*) Rats within litters	9	42·42	4·71	*g*/*h*	1·52 N.S.
(*h*) Error	18	55·79	3·10		—

Significant difference at 5% = ±1·51, 1% = ±2·07, 0·1% = ±2·82.

Conclusions: Diet 205 > 204***, 206*** and 207***;
diet 207 > 204** and 206**;
diets 204 and 206, not significant.

* Significant at 5%. ** Significant at 1%. *** Significant at 0·1%. N.S. = not significant.

the bread a value of 55·8 was obtained, while the two mixtures had b.v. of 57·0 and 61·0 respectively. The latter value but not the former is significantly superior to that of the unsupplemented bread (Table 5). The calculated additive b.v. for the 2 and 6% milk-bread mixtures work out at 56·9 and 58·9. The difference between the calculated and observed values for the bread supplemented with 2% milk is obviously without statistical significance. For the 6% addition the difference amounted to 1·9 ± 0·91 with a probability of 17 : 1 against this difference being due to chance. Our earlier suggestion of a lack of supplementary relationship between bread and milk proteins at levels not exceeding 6% milk is thus confirmed with a more normal sample of dried skim milk.

Exp. 4. *An examination of the biological values and true digestibilities of the proteins of some commercial samples of dried milk*

For reasons given on p. 408 we decided to examine the b.v. of representative samples of English commercial dried skim milks. We were particularly interested in the roller-dried variety, as our abnormal sample was made by that process and because roller-dried milk is more suitable for bread making. Through the kind offices of Prof. E. Capstick, of the Ministry of Food, we obtained fifteen different samples which were manufactured by thirteen firms mostly during the early summer of 1942. These samples were examined for colour, flavour, odour, *p*H and titratable acidity after reconstitution, percentage of ash, and for the alkalinity of the ash by the Tillmans-Bohrmann method (23). Seven of the samples contained appreciable amounts of neutralizer, and four, including one of the neutralized samples, were unusually dark in colour. As it was impracticable to test all of the samples biologically, three representative samples were selected for this purpose. These were a high-grade sample 'A' of normal composition, from unneutralized milk; a sample 'B' of normal composition, from unneutralized milk, but of very dark colour and abnormal odour, indicative of overheating during drying; and a sample 'C' of abnormal composition made from heavily neutralized milk, of normal colour but with an objectionable odour.

The analytical data were as follows:

	A	B	C
*p*H	6·62	6·58	7·28
Titratable acidity, as lactic acid (%)	1·89	1·88	0·11
Ash (%)	8·14	7·83	10·07
Alkalinity of the ash	Normal	Normal	Very high

None of the fifteen samples showed the combination of faults exhibited by the sample which gave rise to the inquiry.

Table 6. *Exp.* 4. (1) *Mean biological values and true digestibilities for commercially dried milks.* (2) *Analysis of variance of the biological values*

(1)

Type of milk...	Biological value				True digestibility			
Period	'House-hold' Diet 200	Good roller-dried Diet 201	Over-neutralized Diet 202	Over-heated Diet 203	'House-hold' Diet 200	Good roller-dried Diet 201	Over-neutralized Diet 202	Over-heated Diet 203
1	88·4	86·8	83·1	79·5*	91·1	91·5	88·3	85·6*
2	79·8	86·8	81·1*	79·2	89·1	88·9	89·9*	89·7
3	72·3	80·3*	76·5	73·9	90·7	88·3*	89·1	88·9
4	76·4*	71·9	73·0	68·3	91·3*	91·2	90·4	90·1
Pooled estimate	78·6	80·9	78·2	76·2	90·5†	90·1†	89·4†	88·8†
Range	51·7–93·2	63·0–97·3	63·9–91·2	58·5–84·8	81·1–96·1	83·8–94·9	82·9–91·9	84·4–91·8

* Two values only—one rat died of pneumonia during the first experimental period.

† Mean for eleven values.

(2)

Component	D.F.	Sum of squares	Mean square	Variance ratio
(*a*) Total	43	2749·39 (+litter s.sq.)		
(*b*) Litters	2	Not determined		
(*c*) Periods (*d*) Periods × litters	9	1487·03	165·22	$cd/h = 4{\cdot}92^{**}$
(*e*) Diets (*f*) Diets × litters	9	416·68	46·30	$ef/h = 1{\cdot}38$ N.S.
(*g*) Rats within litters	8	342·30	42·79	$g/h = 1{\cdot}28$ N.S.
(*h*) Error	15	503·38	33·56	—

** Significant at 1%. N.S. = not significant.

Analysis of variance. Each litter was analysed separately (diets, periods, rats and error), the third litter, where all values for one rat were missing, being treated as a Latin square with one missing row(28). Corresponding terms were then pooled. This gives an unbiased estimate of error. Variance of rats within litters is given directly, but periods and diets are combined with their interactions with litters. To complete the analysis, we need the litter variance (2 degrees of freedom), but as litters are not balanced, the usual method of calculation would be invalid.

Significant difference. The measure of treatment effect in litter 3 is $(3T + C)/8$ (in general, for a $p \times p$ square, $(p-1)\,T + C/p\,(p-2)$, Yates(28)), where T denotes the treatment total and C the total of the column (period) containing the same missing value. This measure is not directly comparable with the treatment means of litters 1 and 2; the mean treatment measure exceeds the general litter mean in the ratio 3 : 2 (in general, $(p-1) : (p-2)$). The pooled treatment effect was therefore estimated by averaging the treatment means of litters 1 and 2, and $\frac{3}{4}$ of the treatment measure of litter 3. The standard error of a difference between two treatment means in the complete litters being $\sqrt{(2\sigma^2/p)}$ and between two treatment measures in litter 3 $\sqrt{[2(p-1)\,\sigma^2/p(p-2)]}$ (with $p = 4$), we obtain for the pooled treatment effects a significant difference $\frac{3}{4}\sqrt{(\frac{1}{2}\sigma^2)} \times t$, where σ^2 has been estimated as 33·56 with 15 degrees of freedom. Significant difference at 5% = ±4·75. Therefore none of the differences between diets is significant.

For comparison, household milk, a spray-dried product made in the United States (*p*H 6·62, ash 7·95%), was also assayed.

The composition and analysis of the diets is given in Table 1 and the results in Table 6.

Results

The household milk yielded a b.v. of 78·6 and the good-quality roller-dried milk one of 80·9. These values may be compared with values reported by us previously for spray-dried whole milk, 84·0(24); spray-dried skim milk, 90·3(25) and 88·5(15); and for roller-dried whole milk, 83·3(24), all freshly prepared. Fairbanks & Mitchell(14) reported for choice commercial skim milk dried by the roller process and for pre-heated spray-dried milk values of 81·6 and 82·3 respectively.

The over-neutralized sample gave a value of 78·2 and the overheated sample one of 76·2. It will be seen from Table 6 that the differences in b.v. between these representative samples, though suggestive, lack statistical significance.

The values for true digestibility differed only slightly between themselves, the lowest, 88·8, was that for the overheated sample. It may be recorded that the lowest value observed by us in twenty-one separate assays of various types of milk on young rats was that of 85·2 for the abnormal sample with a b.v. of 55·2 (cf. p. 404); Fairbanks & Mitchell(14) reported 81·4 for their scorched roller-dried skim milk.

SUMMARY

1. A sample of roller-dried skim-milk powder, found to possess the very low biological value of 55·2, supplemented slightly but definitely the proteins of national wheatmeal bread when it supplied 23% of the total nitrogen. No such effect was observed with another sample of milk with a biological value of 71·1 when it supplied 17% of the total nitrogen, or with a further sample of milk with a biological value of 81·3 when added to bread at a 6% level supplying about 15% of the total nitrogen. On the first two occasions the biological value for two different samples of national wheatmeal bread was 48·9 and 55·0 respectively for young rats; for the same rats when mature the values were 55·8 and 64·6. No differences attributable to age were found with milk. Baking did not affect the biological value of the proteins of the milk added to the flour.

2. The biological value of a sample of spray-dried skim milk declined from a value of 88·5, observed 18 months after manufacture, to 71·1 some 36 months later. The true digestibility remained unchanged.

3. Commercial samples of spray-dried and roller-dried skim milk of good quality, and of over-neutralized and overheated roller-dried skim milk, yielded biological values of 78·6, 80·9, 78·2 and 76·2 respectively and true digestibilities of 90·5, 90·1, 89·4 and 88·8 respectively.

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344. A NOTE ON CERTAIN VITAMINS OF SOW'S COLOSTRUM

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As far as we know little has been published on the vitamin content of sow's colostrum or milk apart from information dealing with vitamin A(1, 2). In the present report data are given for the concentration of vitamin A, vitamin B_1, riboflavin and vitamin C in samples of colostrum from eighteen lactations by eleven sows.

EXPERIMENTAL

Feeding and management of the sows. All the sows were of the Large White breed. The routine in the piggery is to allow two farrowings a year. Within a week after service the sows are placed in a wood where they have free access to grass. They remain there until about a fortnight before farrowing and are then brought in to a Danish-type farrowing house. The sows receive a balanced proprietary pig meal supplemented during the suckling period with approximately 1% of veterinary cod-liver oil.

Collection of colostrum. Samples were obtained during the first stages of farrowing, about 90 ml. being collected from several teats by manual expression into a brown glass bottle within an hour of the onset of parturition. The specimens were brought to the laboratory as soon as possible, placed in the ice-chest, and analysed without delay.

Analytical methods. Fat was measured by the Gerber test as described by the British Standards Institution(3) and total solids by the method of Golding(4). The methods used

for measuring vitamin A, vitamin B_1 and riboflavin were as described in a previous publication (5). For the estimation of vitamin C, 10 ml. of a precipitating mixture were added to 5 ml. of colostrum. The precipitant consisted of 4 parts of a mixture in the proportions of 3 : 2 of saturated ammonium sulphate solution and 30% trichloroacetic acid, and 1 part of 10% sodium metaphosphate (6) solution.

After shaking, the precipitated colostrum was allowed to stand for 20 min., when, after filtration, ascorbic acid was titrated with the dichlorophenol reagent.

Table 1. *Certain constituents in sow's colostrum*

Sow no.	Lactation	Date of farrowing	Total solids (g./100 g.)	Fat (g./100 g.)	Vitamin A		Vitamin B_1 (μg./100 ml.)		Riboflavin (μg./100 ml.)	Reduced vitamin C (mg./100 ml.)
					i.u./100 ml.	i.u./g. fat	Free	Total		
2696	2	9. xii. 42	26·0	2·5	103	41	—	85	34	—
823	2	12. i. 43	—	1·9	215	113	—	—	—	—
822	2	13. ii. 43	—	—	245	—	—	110	53	25
876	2	17. ii. 43	—	—	105	—	—	126	40	17
824	2	17. ii. 43	—	—	200	—	—	145	40	22
873	2	20. ii. 43	22·7	4·9	180	37	93	145	56	28
874	2	11. iii. 43	22·8	3·7	298	81	100	136	48	32
2696	3	7. vi. 43	21·7	2·7	200	74	—	143	52	25
2697	3	7. vi. 43	24·7	4·7	255	54	—	92	61	29
860	2	9. vi. 43	27·2	4·6	462	100	—	220	21	29
823	3	7. vii. 43	23·6	2·3	305	133	—	161	52	28
2714	3	19. vii. 43	25·5	3·9	280	72	—	260	—	12
822	3	9. viii. 43	20·6	2·5	225	90	—	148	50	28
876	3	10. viii. 43	22·7	3·5	320	91	—	142	31	23
873	3	14. viii. 43	26·3	5·8	305	53	—	185	78	36
824	3	18. viii. 43	23·2	2·8	252	90	140	162	38	28
823	7	27. vi. 45	22·5	1·7	225	132	98	116	42	12
1963	2	18. vi. 45	25·4	3·0	272	91	—	81	36	13
Mean			23·9	3·4	247	83	108	145	46	24
Range			20·6–27·2	1·7–5·8	103–462	37–133	93–140	81–260	21–78	12–36
No. of samples			14	15	18	15	4	17	16	16

RESULTS

The results are set out in Table 1.

Total solids and fat. The variation was large, the range for total solids being 20·6–27·2% with a mean of 23·9%, while the fat content varied from 1·7 to 5·8% with a mean of 3·4%. This variation is, however, no greater than for cow's colostrum (7, 8). It should be emphasized that, in order to obtain a true value for the fat content of milk or colostrum, the udder should be emptied completely. With samples taken in the manner described there is a possibility of marked errors. This criticism undoubtedly applies to all work published on the composition of sow's colostrum. It will, nevertheless, be of interest to note that it appears from our data that the level of fat in the colostrum may be a characteristic of individual sows and that the variations in this respect 'within sows' are smaller than those 'between sows'. Thus the fat percentages for the two lactations of sows 2696 and 873 were 2·5 and 2·7, and 4·9 and 5·8 respectively, while for the three lactations of sow 823 the values were 1·9, 2·3 and 1·7.

The mean value for fat observed by us is lower than that of 5·14% quoted by Hughes & Hart (9) or that of 4·57% given by Albig (10). Hughes & Hart give also an extremely high figure, 31·86%, for the total solids content of colostrum obtained during parturition. Our mean value of 23·9% is much nearer that of 25·20% found by Albig on the first day of lactation. It should be noted that our highest individual value, 27·2%, is still far below the mean value of Hughes & Hart.

Vitamin A and carotenoid pigments. The colostral fat was always almost colourless, even in summer when the sows had access to plentiful grass. The very small amount of pigment obtained from the combined fat from sows 2696, 2697 and 860 was subjected to chromatographic analysis on an aluminium oxide column. Two faint xanthophyll zones were observed but no trace of β-carotene.

The values for vitamin A are given in the table per 100 ml. and also per g. fat. The latter figures would not be affected by possible errors in sampling for fat. The mean value per 100 ml. colostrum is 247 i.u. with extremes of 103 to 462 i.u. These values are of the same order as those of Benham (2) which ranged for five samples from 131 to 254 i.u./100 ml. It should be noted that Benham's sows were 'at least partly deficient in vitamin A at parturition'. For his sixth sow which received 400,000 i.u. of vitamin A during the 10 days prior to farrowing the value was 1165 i.u./100 ml. A few values are also given by Schofield *et al.* (1) who, unfortunately, do not state the volume of colostrum to which their figures refer. The values range from 42·8 to 97·5 i.u. for samples from three sows receiving ordinary rations; for two sows which were given daily, during pregnancy, 30,000 i.u. of vitamin A the figures were respectively 571 and 850 i.u.

Our values for the vitamin A potency of sow's colostrum are on the whole lower than those for the colostrum of other farm animals. Our own data (11), for example, for thirteen samples of Shorthorn colostrum range from 820 to 8160 Moore blue units and from 411 to 2026 Moore yellow units per 100 ml., or, if the conversion factors of 0·375 and 0·73 required under our experimental conditions to convert respectively Moore blue and yellow units to international units are applied, 308 to 3060 i.u. as vitamin A and 300 to 1480 i.u. as carotene per 100 ml. Stewart & McCallum (12) reported a mean value of 392 i.u. for the total vitamin A activity of 100 samples of colostrum from Ayrshire cows with a range of 35 to 1181 i.u./100 ml. Biological tests on rats carried out by American workers yielded values from 1600 to 2800 i.u./100 ml. for the total vitamin A activity of first day's colostrum (13).

For the colostrum of cross-bred ewes Underwood & Curnow (14) recently gave values for the total vitamin A activity ranging in one year from 988 to 1608 i.u./100 ml. with a mean of 1287 i.u./100 ml., but in the following year, which was exceptionally dry, the values ranged from 261 to 717 with a mean of 402 i.u./100 ml. In this country Barron (15) has measured vitamin A in thirteen samples of colostrum from Dorset Horn cross Border-Leicester ewes. His values are expressed in blue units, but as his method of carrying out the measurements in the Lovibond Tintometer was the same as ours (Barron, private communication), the factor used by us for the conversion of Moore blue units to international units can be applied. This calculation gives a mean value of 230 i.u./100 ml. which is of the same order as our figure for sow's colostrum. The data of Sattersfield, Clegg & Holmes (16) for Hampshire ewes are expressed in units insufficiently defined to allow conversion by us to international units.

Vitamin B_1. The mean value for seventeen samples is 144·5 μg./100 ml. with a range of 81–260 μg./100 ml. This is about double the concentration found previously in cow's colostrum (17).

On four occasions, the free vitamin B_1 was measured at the same time as the total. The figures show that the amount of co-carboxylase present in the early colostrum of the sow is much less than that found in cow's colostrum at a corresponding stage of lactation (17).

Riboflavin. In the sixteen samples examined, riboflavin averaged only 46 μg./100 ml. with extremes of 21–78 μg./100 ml. Compared with the concentrations of 350–780 μg./100 ml. found by us(17), or concentrations of 420–670 μg./100 ml. reported by Kramer, Gardner, Kunerth & Riddell(18), in cow's colostrum, these values are strikingly low. We do not know at present whether they persist as lactation progresses or whether the concentration rises in later milk. An experiment carried out with young piglets showed, however, that no benefit is derived by suckling young from an addition of riboflavin to their exclusive diet of milk. Thirteen littermate pairs of piglets as uniform as possible were selected from

Table 2. *The growth of suckling pigs with and without a supplement of riboflavin*

			Live weight gain					
			at 1 week		at 3 weeks		at 8 weeks	
Litter no.	Pair no.	Weight at birth	Control	Suppl.	Control	Suppl.	Control	Suppl.
823	1	C 3·4	3·0		10·2		31·6	
Third litter,		S 3·0		3·1		9·5		21·5
born on	2	C 2·5	2·1		8·0		21·8	
7. vii. 43		S 3·1		2·1		7·3		27·1
	3	C 2·8	2·4		8·2		25·5	
		S 2·5		2·4		7·5		19·7
	4	C 2·9	2·6		8·9		28·2	
		S 3·4		2·9		9·6		31·1
876	1	C 3·5	3·2		7·5		19·0	
Third litter,		S 3·4		3·0		8·3		28·1
born on	2	C 3·1	3·0		9·7		26·9	
10. viii. 43		S 3·6		2·9		8·6		25·6
	3	C 3·3	2·4		7·7		27·2	
		S 3·3		2·2		6·9		19·7
	4	C 3·1	1·6		5·5		21·9	
		S 3·6		2·1		5·9		26·9
2714	1	C 3·0	3·0		8·9		31·5	
Third litter,		S 3·4		3·5		9·1		32·1
born on	2	C 2·6	2·9		7·4		24·4	
19. vii. 43		S 2·6		2·9		8·2		21·4
	3	C 2·7	2·5		6·2		27·4	
		S 2·1		2·5		7·2		18·8
	4	C 3·6	2·1		5·2		22·5	
		S 2·5		1·8		4·5		24·9
	5	C 2·7	1·7		4·4		17·1	
		S 1·9		1·6		4·4		20·3
Mean		C 3·0	2·5	2·5	7·5	7·5	25·0	24·4
		S 3·0						

C = control; S = supplemented

litters of three sows when 1 week old. They were allowed to suckle their dams in the usual way but, during the second week of lactation, one member of each pair chosen by lot received daily by mouth 4 mg. of riboflavin. The piglets started taking solid food at 3 weeks. Table 2 gives details of their growth.

Vitamin C. Only the reduced form was measured, as the precautions taken in the collection of the samples made it unlikely that the reversibly oxidized form would be present. The vitamin C content of sow's colostrum proved to be exceptionally high, the mean value for seventeen samples being 23·8 mg./100 ml. with a range of 12–36 mg./100 ml. According to Kon & Watson(19) cow's colostrum contains only about one-tenth of this amount; other authors(20) found that values for fifteen samples of ewe's colostrum ranged from 2·0 to 9·9 mg./100 ml. with a mean of 5·9 mg./100 ml.

Concentrations of vitamin C as high as, or higher than, in sow's colostrum have to our knowledge only been reported for the milk of the guinea-pig(21, 22). The guinea-pig, however, in contradistinction to the cow, sow, or ewe, is dependent on an exogenous supply of vitamin C. It would be of interest to find out the reason for the unusual richness of sow's colostrum in this factor. We do not know whether later milk of the sow is equally rich in vitamin C. We are at present investigating this point.

Summary

1. Eighteen samples of colostrum obtained from Large White sows during parturition were examined for total solids, fat, vitamin A, vitamin B_1, riboflavin and vitamin C.

2. The mean values and ranges for the total solids (fourteen samples) and fat (fifteen samples) were 23·9% (20·6–27·2%) and 3·4% (1·7–5·8%) respectively.

3. The mean vitamin A content of eighteen samples was 247 i.u./100 ml. with a range of 103–462 i.u./100 ml., or 89 i.u./g. fat with a range of 42–137 i.u./g. fat. No β-carotene was detected in any of the samples.

4. The mean vitamin B_1 content of seventeen samples was 145 μg./100 ml. with a range of 81–260 μg./100 ml.

5. The mean riboflavin content of sixteen samples was 45·6 μg./100 ml. with a range of 21–78 μg./100 ml.

6. The mean ascorbic acid content of seventeen samples was 23·8 mg./100 ml. with a range of 12–36 mg./100 ml.

Our thanks are due to Mr A. Wagstaff for the estimation of fat and total solids.

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Note added in proof. Work done since the present experiments were completed and which will be published in detail later, has confirmed the absence of dehydro-ascorbic acid from, and the high antiscorbutic potency of colostrum.

REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION F. MILK-BORNE DISEASES

Introductory

There are signs in this country that a less complacent outlook is being generally adopted to the transmission of diseases by milk, and at the present time this attitude has been characterized by more militant methods in order to obtain a greater degree of pasteurization. The first step towards providing clean and safe milk is the fundamentally important one of proper inspection of dairy farms, but even with complete and adequate inspection, the public cannot be completely safeguarded unless the milk, prior to bottling, is efficiently pasteurized. There is no cogent evidence that pasteurized milk is in any way inferior nutritionally to raw milk.

Dolman(1), in a paper on the present status of milk-borne hazards, believes that the actual incidence of disease is higher than that recorded, but no very close estimate of the former in terms of the latter can be made owing to the many weak or missing links in the chain of evidence. Further, he believes that the incidence of milk-borne disease may be decided for all practical purposes by the following three main groups of variables:

(1) The distribution among dairy cattle of tuberculosis, Bang's disease and of streptococcal and staphylococcal mastitis.

(2) The extent to which handlers of milk and milk products may be infected with, or may carry, micro-organisms of the typhoid-paratyphoid-dysentery group, Group A streptococci, enterotoxigenic staphylococci, and diphtheria bacilli.

(3) The extent to which supplies of milk and milk products are effectively pasteurized.

Marked fluctuations in any component of these groups of variables will inevitably be reflected in the apparent trend of the incidence of milk-borne disease. Ravenel(2) has given a brief historical résumé of milk-borne disease, and concludes that on the basis of what has been already proved, further argument for pasteurization seems unnecessary.

Measures to improve the quality of the nation's milk supply have been envisaged in the White Paper(3) published in 1943. The first essential requirement is an increased veterinary inspectorate so that herds can be examined more frequently. It is further suggested that the whole supervision of milk production should be taken out of the hands of local authorities and placed under the veterinary services of the Ministry of Agriculture. This administrative change is to apply to England and Wales, but not to Scotland. The consumption of T.T. milk is to be encouraged by removing the premium paid to producers, and by selling this grade of milk only slightly in excess of the price paid for ordinary milk. It is proposed to schedule certain areas where it will be an offence to sell milk unless it is (1) heat-treated, (2) lawfully sold as T.T. milk, and (3) accredited milk from an accredited herd. It is proposed to apply this control to areas in which there are more than 10,000 inhabitants. Pasteurizing plants may be installed by local authorities. The Government's proposals have, however, been the subject of considerable criticism in the medical press. For instance, a leading article in the *British Medical Journal*(4) states that the health officers are to be deprived of their slowly evolved powers over milk production, which, it appears, are to pass into the hands of veterinary officers, who are not primarily concerned with human diseases or with the preventive steps appropriate for the control of most of them. The medical officer of health will be still responsible in theory for the prevention of these diseases, but in practice he will enter the field only after damage has been done. It has been said that certain authorities have been neglectful of their responsibilities, but it does not appear that the Ministry

of Agriculture has a sufficiency of staff to undertake such a vast amount of work. If it is established that certain small local authorities are neglectful of their duties in regard to milk inspection, then the remedy should be the creation of enlarged areas of Local Government. Finally, the 1941 regulations(5) permit local authorities to grant licences to use the special designation 'pasteurized' in respect of milk, processed also by the high-temperature, short-time method.

Savage(6), in a paper on a national milk policy, again points out the necessity for obtaining a milk supply of the highest quality. As control of bovine disease is a veterinary problem of such magnitude, reduction in the incidence of infection rather than immediate eradication should be aimed at. As there is, in addition, a possibility of contamination during production, all milk should be made safe by pasteurization. Further, the author directs attention to the necessity for providing proper safeguards and adequate supervision in the production and distribution of milk. Pasteurization should be applied to all grades of milk, including T.T., so as to guard against the spread of infection.

Recently published books

A number of books have been published dealing mainly with pasteurization, both from the point of view of the elimination of milk-borne infections, and also from the point of view of the mechanical and technical processes involved. First and prominent amongst these is *The Pasteurization of Milk* by Wilson(7). This work contains a foreword by the Chief Medical Officer of the Ministry of Health, who describes its origin. Prof. Wilson had submitted a report on milk-borne diseases to the Ministry of Health in 1939, but owing to war conditions this had not been published. This report was then brought up-to-date by Prof. Wilson and published privately. In his preface, Prof. Wilson describes the purposes for which the book was written, one such purpose being to record the frequency with which raw milk is infected with various pathogenic organisms, and the amount of diseases for which it is responsible in Great Britain. In chapters 2–4 of this book the reader will find an up-to-date account of the mode and extent of infection of raw milk with pathogenic bacteria, the incidence of milk-borne disease in the human population, and an account of methods of rendering milk safe.

Enoch(8), who is a mechanical engineer, discusses *This Milk Business* from many points of view, and one chapter of the book is devoted to bacterial infection and contamination. This section contains a variety of facts and suggestions of little importance from the medical viewpoint. He advocates the 'in-bottle' method of pasteurization as the most effective means of overcoming any possibility of contamination.

Hill(9) writes as a sanitary inspector in *Pasteurisation*. The second chapter in the book presents the evidence for the necessity of pasteurization, and the author states that since the beginning of this century over 200 outbreaks of epidemic disease due to milk have been recorded. The best sections of this book are devoted to technical matters connected with pasteurizing plants and the methods of checking the efficiency of such plants.

Streptococcal infections

Since the last review of milk-borne diseases, not many outbreaks of epidemic streptococcal infections have been reported in the literature. Further, little has been added to our knowledge of the classification of this group of organisms.

Lancefield's Group A strains are, of course, the main cause of human streptococcal infections, and the infrequent cause of infections in bovines. Allison(10) examined a total of 1283 strains of haemolytic streptococci isolated from throat swabs of human cases of acute upper respiratory tract infections, with the following results:

Streptococcus group	A	B	C	G	Total
No. of strains	1148	1	96	38	1283
Percentage	89·48	0·08	7·48	2·96	100

Stableforth(11) lists the agents of streptococcal mastitis as follows:

Kinds of mastitis	Bacteria concerned	Outstanding features
(1) Common chronic streptococcal mastitis	*Str. agalactiae*, *Str. mastitidis*, Group B (Lancefield), Mastitis Group I	Widespread, incidence 35 % of milking cows; many carriers or mild cases, but causes more clinical mastitis and loss of milk than any other
(2) More acute streptococcal mastitis	*Str. dysgalactiae*, Mastitis Group II	Sporadic, acute or subacute, few carriers, serious in some herds
,, ,,	*Str. uberis* Mastitis Group III	Sporadic, usually subacute, some carriers
,, ,,	*Str. pyogenes equi*, 'Animal *pyogenes*', Group C (Lancefield)	Uncommon, acute, carriers rare
,, ,,	*Str. pyogenes*, Group A (Lancefield)	Rare, acute, carriers rare

Again, Isaac & Nussbaum(12) examined milk samples for haemolytic streptococci, with the following results:

		Streptococci isolated							
		Group A		Group B		Group C		Other	
Milk	No. of samples	No.	%	No.	%	No.	%	No.	%
Grade A	27	0	0	18	66·7	62	22·0	3	11
Grade B	64	0	0	36	56·2	16	25·0	12	19

It is evident, therefore, that mastitis due to Groups A and C are rare, but do on occasion produce an acute infection; on the other hand, the incidence of Group B strains is widespread. Again, infections in human beings with Group B strains are even more rare than with Group C strains. Lancefield's(13) work seems to indicate that Group B streptococci may be associated with both human and bovine infections, while, on the other hand, Simmons & Keogh(14) believe that the human and bovine strains which have the serological characteristics of Group B are possibly distinct in their pathogenic affinities.

Allison(10) has summarized the reports which have been published on infections with Group B strains as follows:

Human infections with haemolytic streptococci belonging to Group B

Authors	No. of cases	No. fatal	No. with endocarditis	No. associated with pregnancy
Congdon (1935)	1	1	?*	1
Lancefield & Hare (1935)	7†	0	0	7
Colebrook & Purdie (1937)	1	1	1	1
Coffey (1937–8)	3	2	?*	2
Fry (1938)	3	3	2	3
Hill & Butler (1940)	12‡	4	2	12
Pomalés-Lebrón (1940)	1	1	?*	1
Rosenthal & Stone (1940)	1	1	1	1
Ramsay & Gillespie (1941)	16§	2	2	16
Fry (unpublished)	4	2	1	3
Total	49	17	9	47

* No data available. † All minor infections. ‡ Including some minor infections.
§ Genital tract sepsis in thirteen cases, none severe.

It will be noted that infections with Group B strains mostly occur in women.

An account of an interesting outbreak in Scotland of scarlet fever due, originally, to a milk infection is recorded by Douglas *et al.*(15). This outbreak occurred in the burgh of Elgin which had a population of 9000, and between 13 January and 2 March 1939, 214 cases of scarlet fever were reported. Notifications of scarlet fever arrived in increasing numbers during the third and fourth weeks of January 1939. The peak day of the epidemic was 26 January 1939, when thirty-one cases were reported. Investigations made in the home of each patient showed that one factor

only was frequently associated with the disease, namely, the consumption of milk from a supply which was designated A. Milk supply A was drawn from cows in a registered herd at one farm. The cows were kept under excellent conditions, and were milked mechanically and, as is customary, the udders were 'stripped' by hand. The herd and the milking arrangements were under the management of one family, with the help of a man who lived apart. Four members of the family had suffered from an illness, two were found to be desquamating, and one had a definite scarlet fever. Of 100 cows in the herd, sixty-eight were in milk, and only one of these showed an enlarged mammary gland. The sixty-seven apparently healthy cows were divided into eight groups, and milk samples were collected. Separate specimens were taken from the cow with mastitis, and from three more in the 'dry' group who were also suffering from clinical mastitis. Bacteriological examination showed that the four individual specimens did not contain haemolytic streptococci, but one of the group samples taken from the apparently clinically healthy animals contained them. The milk from the positive group of cows was excluded on 2 February 1939, and a sample from each cow examined separately. One cow only gave haemolytic streptococci, and a further series of examinations showed that these were being excreted from one quarter only. The cow was kept under observation for a considerable period, and for the first 3 weeks the udder remained normal, but later the affected quarter became definitely indurated.

The cases notified showed typical mild scarlet fever, and it was reported that many persons who did not develop scarlet fever had sore throats, but no estimate can be given of these except that they were large. No deaths could be attributed to the outbreak, but in the later stages of the epidemic an unusually large number of notifications of erysipelas were received. The strain of haemolytic streptococci isolated from the cow, and ten strains isolated from acute cases of scarlet fever, all belonged to Lancefield's Group A and Griffith's Type 3.

Dublin *et al.* (16) published a very interesting paper on milk-borne outbreaks due to serologically typed haemolytic streptococci. They stated that in the 25-year period 1917–41, fifty-seven outbreaks of scarlet fever and sore throat had occurred in the State of New York, exclusive of New York City. They list a series of six outbreaks in foreign countries, and give particulars of nine outbreaks of their own in which the *Streptococcus* was typed. A table has been compiled from the particulars given in their paper:

Milk-borne outbreaks due to serologically typed haemolytic streptococci

Author	Year	Place	Disease	No. of cases	Griffith type	Mode of infection
Dublin *et al.*	1935	Baldwinsville, N.Y. State	Sore throat	500	27	Cow's udder
,,	1936	Wellsville, N.Y. State	Scarlet fever	201	3	,,
,,	1936	Owego, N.Y. State	,, ,,	532	15–17	,,
Camps *et al.*	1936	Chelmsford, England	,, ,,	1600	2	,,
Watson	1937	Doncaster, England	,, ,,	364	2	,,
Henningsen & Ernst	1938	Mørkøv, Denmark	,, ,,	100	Not typable	,,
Leusden & Blitz	1939	Pinneberg, Germany	,, ,,	450	5	,,
Henningsen & Ernst	1939	Vegle, Denmark	,, ,,	128	3	,,
Samson	1939	Romford, England	,, ,,	18	2	,,
Dublin *et al.*	1940	Waddington, N.Y. State	Sore throat	48	19	? direct infection
,,	1940	Kirkwood, N.Y. State	Scarlet fever	6	Not typable	Cow's udder
,,	1941	Pultney, N.Y. State	,, ,,	33	3	,,
,,	1941	Huntington, N.Y. State	Sore throat	116	11–12	,,
,,	1942	Cannonsville, N.Y. State	Scarlet fever	44	3	,,
,,	1942	Coxsackie, N.Y. State	Sore throat	200	2	,,
Douglas *et al.*	1942	Elgin, Scotland	Scarlet fever (Sore throat, not numbered)	214	3	,,

Griffith Type 3 has been identified more frequently than any other single type, being encountered in four out of the fifteen outbreaks. In the nine outbreaks in the State of New York, five were of scarlet fever and four had cases of both scarlet fever and sore throat, and altogether 1650 individuals were involved. With one exception, all were proved to be due to infection of a cow's

udder. In one outbreak, part of the infected milk supply had been converted into Cheddar cheese for export, and in order to make certain that it would not be infective it was treated by a process in which it was heated to a temperature of over 170° F. for several minutes. The various reports leave little doubt as to the method by which milk becomes infected. It is not a question of a human carrier contaminating a milk supply, but the human carrier or convalescent cases infecting the cow, causing some degree of mastitis, and the haemolytic streptococci are then excreted in such large numbers as to cause an epidemic, which is only limited by the amount of milk which has been bulked and distributed.

Diphtheria

Outbreaks of milk-borne diphtheria are comparatively rare. Armstrong & Parran(17) collected the findings in twenty-six outbreaks which had occurred in the U.S.A. between 1906 and 1926, while Wilson(18) collected similar details about thirteen outbreaks occurring in Great Britain between 1912 and 1931. Goldie & Maddock(19) described a small outbreak in Yorkshire. In April 1942, at one farm, three out of four children had developed diphtheria, and later the mother was found to be a carrier. All were removed to the local isolation hospital. The small farm produced 10 gal. of milk per day, part was distributed to three families, and the rest was bulked with other supplies and distributed to 300 houses in a small town. In July, after the children and mother had returned from hospital, seven more cases were reported, and four of these occurred in the three families who received the direct supply from the farm. This milk supply became suspect, and *B. diphtheriae gravis* was actually isolated from the milk on two occasions, while the patients were also infected with the same type of organism. The authors conclude that the isolation of *B. diphtheriae* from milk is considerably simplified by the use of a medium containing potassium tellurite, and that in this particular outbreak diphtheria bacilli gained access to the milk from the throat of the farmer's wife, who was still a carrier.

Staphylococcal food poisoning

Outbreaks of staphylococcal food poisoning continue to be reported, and the ubiquitous presence of staphylococci in the tissues of human beings and animals must provide many opportunities for contamination of foodstuffs and, where the conditions are suitable, for the production of the enterotoxin which is the essential cause of the food-poisoning symptoms. Dolman(1) believes that staphylococcal mastitis is of much greater importance from the point of view of public health than streptococcal mastitis. He states that enterotoxigenic strains, when cultured on sheep-blood agar, show a wide zone of haemolysis, and the presence of this characteristic may be deemed valid evidence of their probable capacity for producing food poisoning. In a series of examinations of milk samples carried out in his department, staphylococci were found as follows:

	Raw milk	Pasteurized milk
No. of samples examined	74	61
No. showing toxigenic staphylococci	64	1

It is obvious, therefore, that efficient pasteurization would largely eliminate this potential pathogen.

Cultural and biological tests do not give any clear-cut differentiation between strains of staphylococci likely to cause food poisoning and those which are not. Attempts have been made to devise culture media which would help. In the first instance, the examination of milk for staphylococci presents certain difficulties, and in order to overcome them, Beveridge(20) evolved an alkaline blood agar. The basic nutrient agar which he prepared had a *p*H of 9·0–9·2, and to each 12 ml. he added 0·6 ml. of unclotted blood. He considers that by this method he can detect more haemolytic staphylococci than with the ordinary blood agar, since the alkaline reaction of the medium prevents the growth of many micrococci other than staphylococci. He believes also that growth on this medium may also be a useful indication of pathogenicity and of origin. Wilson(21) suggested that food substances suspected of containing food-poisoning staphylococci should be cultured in broth for 24 hr., and then 10% sheep-blood agar plates inoculated from the broth.

Thereafter strains are examined for the production of α- and β-haemolysins and for the production of enterotoxins. Again, Barnett(22) reported on the examination of milk samples for staphylococci by plating on phenol-red, brom-thymol-blue, and beef-infusion rabbit-blood agar. Strains were tested for pigmentation, coagulase production, haemolysis, mannitol fermentation, and colour of growth as suggested by Chapman. Approximately one-third of the samples tested were found to contain potential food-poisoning staphylococci.

Bayliss(23) undertook an extensive study of the pharmacological action of the enterotoxin. Its action was tested on young and adult cats, and these experiments showed (1) that it had no direct action on isolated intestinal strips; (2) that emesis resulted following intravenous, intracardial and intraperitoneal injections, but failed to appear subsequent to oral, subcutaneous, or intramuscular administration; (3) that emesis occurred following coeliac ganglionectomy, gastrectomy, spinal cord transection at T2 and unilateral vagotomy; (4) that mild retching movements and rarely emesis resulted subsequent to enterotoxic injections following (*a*) double vagotomy, (*b*) abdominal evisceration, and (*c*) spinal cord transection at C7; (5) that emesis never occurred following (*a*) destruction of the vomiting centre, (*b*) injection of enterotoxin into the fourth ventricle over the vomiting centre, and (*c*) transection of the central nervous system between the anterior border of the pons and the posterior border of the hypothalamus; and (6) that morphine inhibited, ergotoxin inhibited or delayed, while atropine and pentobarbital had little or no effect on emesis due to the enterotoxin injection. Finally, the author concluded that the action of staphylococcal enterotoxin on peripheral sensory structures is of greater importance in the initiation of emesis than the direct action of the toxins on the vomiting centre.

Wilson(18) believes that while various cultural tests are required to help to identify a toxigenic *Staphylococcus* strain, the kitten test will show whether or not enterotoxin has been produced. Thus in a food-poisoning outbreak at Hamilton, Ontario, in 1939, the positive kitten strains from the pie were identical in all their toxin characteristics with the positive kitten strains isolated from the nose and throat of the persons in charge of the preparation of pies. The lethal, necrotic and lytic toxins can be destroyed in three ways before testing for enterotoxins by incubating for several days with 0·3% formalin, by boiling filtrates for 20–30 min., or by mixing with an antitoxin that selectively neutralizes these toxins.

Dolman & Wilson(24) believe that when an outbreak of food poisoning occurs with the typical symptoms of *Staphylococcus* food poisoning, and when staphylococci are recovered from the suspected food in fairly large numbers, the condition can be reasonably ascribed to enterotoxigenic staphylococci. Further, a positive kitten test does give definite evidence that a strain does produce an enterotoxin, and that the same kittens may be used on several occasions provided that an interval of 7–10 days is allowed to elapse between the injections. Hammon(25) has introduced a modified cat test for enterotoxin. He found that for all practical purposes, the same amount of toxin given intravenously can be expected to produce a similar response in any animal ranging from about 800 g. to the largest cat. To carry out the test, the cat is placed on its back on an animal board, and the inner aspect of one thigh is shaved to a point just below the knee. Pressure in the inguinal region causes the saphenous vein to stand out, and a 26-gauge needle can be used for the inoculation. The toxin to be tested is first heated to 100° C. for 30 min. in a water-bath, and after the material has been allowed to cool and to precipitate, 0·5–5 ml. can be injected. After repeated inoculations, tolerance develops, but this occurs to a much less degree than when the intra-abdominal route is used. The author gives details of an experiment designed to demonstrate the effect of repeated inoculations as follows:

Effect of a 3·0 ml. intravenous test dose of enterotoxin on cats with varied experiences after a 60-day rest period

Cat	No. of inoculations	Time in min.: nausea noted	Time in min.: vomiting occurred	Degree of diarrhoea
1	1	39	40	+ + + +
2	2	38	40	+
3	2	55	58	+
4	6	63	—	0
5	7	55	—	0
6	10	—	—	0

The effects of enterotoxin given by the intravenous method are similar to those described by Dolman, Wilson & Cockcroft(26) for the intraperitoneal test. In usually about 30 min. nausea and vomiting occur; coarse tremors are noted and the hair stands erect, probably due to a rigor, as the temperature may rise to 104 or 106° F.; diarrhoea is usually marked, but may be of a mild nature; the cat improves within 3 or 4 hr., and is usually practically normal within 24 hr. The author notes that diarrhoea may occur after the injection of control material, and that vomiting is really the only symptom which can be assigned specifically to the toxin. The nature of the enterotoxin was investigated by various chemical and immunological methods, and the conclusion is reached that probably the active principle is in the form of a large complex carbohydrate molecule and is not a protein. Dolman(27, 28) described a number of human experiments which were designed to differentiate between the various types of staphylococcal toxins. Toxins were obtained from strains of staphylococci which produced α and β toxins and β toxins only. When these toxins were fed to human beings no ill effects were caused. When, however, a filtrate containing α toxin and enterotoxin was given to volunteers, then the typical symptoms were produced. The results of the experiments confirmed the view that the enterotoxin is different from β toxin, but both types of toxin may cause reactions when given to kittens.

Measures to prevent staphylococcal food poisoning have been suggested. Thus, Stritar, Dack & Jungewaelter(29) and Korff(30) reported that custard-filled pastries may be reheated without spoiling the flavour or appearance of the product. Gilcreas & Coleman(31) thought it advisable to reinvestigate the whole position, and carried out experiments to determine the time and temperature of rebaking necessary to kill an enterotoxigenic strain of *Staphylococcus* in the custard filling in eclair shells, and to examine the palatability of such rebaked cream-filled pies. After numerous experiments they concluded that staphylococci in custard filling in eclair shells were rendered non-viable by heating for 15 min. at 216–220° F., and that rebaking did not impair the appearance or palatability. The use of freshly prepared custard and prompt rebaking are essential to ensure the absence of preformed thermostabile toxins, and the adoption of this procedure should greatly reduce the incidence of food poisoning. Coughlin & Johnson(32) note that the legal requirements of the sanitary code for the State of New York do not make any special provision for the prevention of outbreaks of food poisoning due to cream-filled cakes. The authors point out, however, that the Westchester County Department of Health require that all custard must be heated, when prepared, to at least 200° F. for a period of 10 min., and then placed in a refrigerator 500° F. within an hour. Other American Health Departments have been urging bakers to rebake cream-filled pastry, and the American Institute of Baking has brought to the notice of its members the work of Stritar, Dack & Jungewaelter.

A small outbreak of staphylococcal food poisoning was reported from New York State(33), and involved seven persons in two related households. Cupcakes containing a cream centre had been held at room temperature for 55 hr. after filling, and were then eaten. The usual symptoms of staphylococcal food poisoning developed, and *Staphylococcus aureus* was isolated from one of the remaining cakes. Fingherov(34) described an outbreak in which the food involved was cream tarts, and from which *St. aureus* was obtained in almost pure culture. Investigation revealed that the pastrycook who made the tarts was suffering from a burn of the second degree of the hand. This was discharging pus, and from it an identical *Staphylococcus*, as shown by pigment formation, biochemical characteristics, and toxin production, was obtained. Coughlin & Johnson(32) reviewed the records of the New York State Health Department during the 5-year period 1935–9, and showed that out of a total of thirty-one outbreaks of food poisoning, seventeen, involving 1246 cases, were believed to have been caused by cream-filled baked goods. *St. aureus* was found in the suspected food in nine of the outbreaks caused by cream-filled cakes, and a toxin was demonstrated in three. Getting, Robertson & Foley(35), in a paper on sources of food-borne poisoning outbreaks in war industry, record a series of eighteen such incidents, in four of which staphylococci in ice cream and cream fillings were the etiological agent.

Macdonald(36) has reported a small outbreak which was due to cheese made from goat's milk. Five people had tea shortly after 6 p.m. one evening, and between 10 and 11 p.m. four had symptoms of diarrhoea, abdominal pain, and dizziness. From the various specimens examined,

the only organism isolated of any significance was a *Staphylococcus*. This was obtained from the faeces of one patient, and from four samples of the cheese. The milk from the goat was also found to contain 200 staphylococci per ml. The three strains of *St. aureus* from the cheese, goat's milk and faeces of one patient all gave similar biochemical reactions, and were coagulase positive. Culture filtrates of the cheese strain produced severe abdominal pain and diarrhoea in a human volunteer, but when these filtrates were boiled they produced no ill effects, which is contrary to what is usually found when enterogenic toxin is present. Weed *et al.*(37) described a family outbreak. The father was taken ill with acute enteritis, and during the next day two children became acutely ill and both died within 24 hr. These three members of the family had all consumed raw milk obtained from a goat which was also ill and which died. It was found that the goat was suffering from an acute staphylococcal mastitis. The *Staphylococcus* obtained from the udder was identified as a haemolytic *St. aureus* capable of producing the typical enterotoxin.

Bovine tuberculosis

During the past century there was a rapid decline in the death-rate from tuberculosis. In 1851–5 the death-rate per million was 3638 for all forms of tuberculosis, and 2890 for respiratory tuberculosis, while in 1938 the corresponding rates were 602 and 476. During the last war there was a considerable increase in the incidence of the disease, particularly in young adults, and this increase was still manifest for a few years after its termination. With the advance in our knowledge of nutrition and hygiene it was thought that a war-time increase would not be so prominent. However, such an increase has occurred, and the death-rate has increased considerably. Thus Stocks & Lewis-Faning(38) estimate that had the incidence of new cases continued to decline as in the pre-war period, some 6000 fewer deaths would have occurred during 1940–3 from respiratory tuberculosis, and that in England and Wales, owing to a temporary rise in short-term fatalities during the severe conditions of 1940–1, some 2500 notified patients suffering from pulmonary tuberculosis died in those years instead of in 1942–3. Another 1500 notified persons, and about 1000 unnotified persons, who in normal circumstances would not have died of the disease, must have died of respiratory tuberculosis during 1940–1.

The Medical Research Council's report of the Committee on Tuberculosis in War-time(39) once again stresses the need, amongst other measures, of reducing the spread of bovine tuberculosis by the provision of a safe milk supply. Attention is called to the fact that at least 40% of cattle throughout the country react to the tuberculin test, although there is great variation in the number of reactors in dairy cattle in different parts of the country. Matthews(40) states that in certain districts in Great Britain it may reach 100%; Montgomorie & Rowlands(41) found 11·8% reactors in a north Wales district, Thomas(42) 2·6% in south Wales, and Rabagliati(43) 7·6% in part of Yorkshire. At least 6% of supplies from farms contain tubercle bacilli. The war-time evacuation policy transferred large numbers of children from areas in which the milk is pasteurized to areas in which it is not, with consequent increased risk of infection. It is urged, therefore, that pasteurization and the heat treatment of milk should be extended as much as possible, and every effort should be made to provide school and other children with safe milk. It has been estimated that 98% of the milk supply of London, 40–50% in large towns, and 15% in smaller towns is now pasteurized.

There is at present not much evidence that the increase in tuberculosis is due to an increase in bovine infections, but Committees of the Medical Research Council have this point under investigation, both in England and in Scotland. At present the bovine bacillus is responsible for some 30% of cases of non-pulmonary tuberculosis in Great Britain. Griffith(44) stated then that 'the latest findings in regard to this intractable problem should be made widely known, since they prove beyond any doubt that bovine tuberculosis is a serious menace which must be fought with the utmost vigour, and the apathy of public opinion in this respect is astonishing in face of the incontrovertible evidence which has been published during the last quarter of a century'. However, little has been achieved by the introduction, in 1937, by the Ministry of Agriculture, of the Tuberculosis (Attested Herd) Scheme to eradicate tuberculosis from dairy herds.

Gloyne(45) states that Sir W. Savage has estimated that 1% of respiratory, and 23% of non-respiratory tuberculosis, or 5·5% of the total deaths from all forms of the disease at all ages are due to the bovine type of bacillus. When these factors are applied to the Registrar-General's statistics for England and Wales for 1938, the following is obtained:

Deaths from tuberculosis, 1938

		Calculated bovine deaths
Respiratory	21,930	219
Non-respiratory	4,246	976
Total	26,176	1,195

Notified cases, 1938

		Incidence of bovine infections
Respiratory	37,879	378
Non-respiratory	12,810	2,946
Total	50,689	3,324

Wilson(46) compared two methods by which the number of deaths due to the bovine type of bacillus can be arrived at. The first method is that used by the Cattle Diseases Committee(47), and is based on previous studies of the frequency with which the bovine type of bacillus is found in different clinical forms of tuberculosis. It is then possible to calculate, from the Registrar-General's figures, the number of deaths that occur in any one year as the result of infection with the bovine type. The other method suggested by Wilson is based on the fact that 98% of London's milk supply is pasteurized and, it is argued, that, if in London, so many cases of pulmonary tuberculosis correspond to so many cases of non-pulmonary tuberculosis, then so many cases of non-pulmonary tuberculosis will be associated with a known number of cases of pulmonary tuberculosis in England and Wales. Corrections in this calculation have to be made for variations in the distribution of the sexes, in age groups, and in death-rates in different parts of the country. When these two methods are applied, the probable number of deaths from bovine tuberculosis in England and Wales is:

Year	Method 1	Method 2
1931	2285	2207
1937	1603	1916

The difficulties associated with the eradication of tuberculosis from dairy herds has made it necessary to adopt other methods of control. The Government's White Paper(3) on milk policy indicates that areas will be scheduled within which no milk shall be sold unless it is adequately heat-treated, tuberculin-tested, or 'accredited' when coming from a single herd. Unfortunately, it is not always possible to detect tuberculosis of the udder by clinical examination, for usually the lesions are often so small as to be unrecognizable. The procedure at present for the detection of tubercle bacilli in milk is cumbersome and takes a considerable time. Apart from detection by veterinary officers, the usual routine is for the officials of the health authority to collect a sample of milk for bacteriological examination, including animal inoculation, which may take from 4 to 8 weeks. If a positive result is obtained, the medical officer of health of the area notifies the veterinary inspectors of the Ministry of Agriculture, who then proceed to examine the cows in the herd and who may, if the source is not detectable, have to take more group and individual samples for examination. In certain instances it may take anything up to 4 months to eliminate a tuberculous cow from a herd. It is difficult to see how the diagnosis could be expedited, but it seems to be essential that as soon as tubercle bacilli are found in a supply, then that supply should not be allowed to be used in the raw state but should be pasteurized efficiently. So far, there is no legal authority to enforce this.

The results of investigations into the incidence of tubercle bacilli in milk, which have recently been published, are summarized:

Tubercle bacilli in milk

Author	Year of publication	Country	No. of samples	% +
Thomas & Thomas(42)	1940	Wales	1574	1·1(13)
Lorenzen(47)	1940	Germany	2296	1·08
Gloyne(45)	(1928–31)	England, Home	1560	9·1
	(1935–39) published 1942	counties	—	6·2

Although Gloyne's(45) figures suggest improvement up to the outbreak of the war, the figures do not compare favourably with those reported from other countries. In Wales, Thomas & Thomas(42) examined 1190 samples by guinea-pig inoculation from ungraded dairy herds, and found thirteen, or 1·1%, positive, while in a further 439 samples from accredited herds, 2, or 0·5%, were positive.

A study of the epidemiology of pulmonary tuberculosis due to bovine type of bacillus shows that infection can be acquired in three main ways, first, by the alimentary tract through ingestion of infected material, most commonly milk, secondly, by air-borne droplet infection as a result of contact with cattle, and thirdly, by air-borne contact infection with humans also infected with the bovine bacillus. Griffith & Munro(48) have collected the data so far obtained in Great Britain, and this is summarized as follows:

Bovine pulmonary tuberculosis

Authors	Region	No. of cases	Eugonic human	Dysgonic human	Bovine	Percentage bovine
	Scotland					
Griffith & Wang	Edinburgh, Glasgow and Lanark (early cases)	102	100	—	2	2·0
Griffith & Smith	North-east counties	540	468	23	49	9·1
	Aberdeen city	432	399	14	19	4·4
Griffith & Munro	Middle and south	515	476	8	31	6·0
	Additional	15	14	—	1	6·6
	East	1165	1107	—	58	5·0
		2769	2564	45	160	5·7
	England, Wales and Eire					
Cumming	North	888	874	—	14	1·5
Lynn & Cutbill	Middle	1000	977	1	22	2·2
Lynn & Cutbill	Middle	315	305	—	10	3·1
Griffith & Menton	Middle	230	228	—	2	0·8
Griffith	South	694	684	6	4	0·5
Pagel	South	100	99	—	1	1·0
Cumming	South	195	194	—	1	0·5
Cumming	Various parts (selected)	245	224	—	21	0·8
Cumming	Various parts (selected)	Unspecified number (4+)	—	—	4	2·0
Cumming	Wales	203	201	—	2	2·0
Cumming	Eire	320	320	—	—	—
		4194	4106	7	81	1·9

The north-east of Scotland has the highest incidence of such infections, and altogether the incidence in England is much lower than in Scotland. Again, Cutbill & Lynn(49) have brought their work up-to-date, and in the cases admitted to their Cheshire sanatorium, out of 2101 examined, the incidence of bovine tubercle bacilli was forty-eight, or 2·28%. These workers have

tried, as far as possible, to determine the mode of infection in these cases, with the following results:

Probable mode of infection in forty-eight cases of bovine pulmonary tuberculosis

Alimentary	Air-borne cattle contact	Air-borne familial	No evidence obtained	Total
16	10	3	19	48
33%	21%	6%	40%	

Again, Griffith & Munro(48) believe that the anatomical evidence in about one-third of the cases in Scotland, and in a quarter of those in England, was strongly in favour of the digestive tract as the channel of entry of the bacilli. It is difficult to obtain a concrete example of the transmission of infection from man to cattle, but such a possibility has to be reckoned with. Magnusson(50) states that the bovine type of tubercle bacilli was introduced into Sweden about 1800, and in the three years 1936–9 was responsible for 3·3–8·9% of cases reported in man, farm labourers proving particularly susceptible. The bovine type can be transmitted from man to cattle, and an instance is reported where, in a herd of seventy-four cows which had been free from reactors for 7 years, forty-nine suddenly appeared. Infection was traced to a woman milker, who had assisted on the farm for a few weeks, and who was later found to be suffering from pulmonary tuberculosis of the bovine type.

There is not much recent work available on the incidence of bovine tubercle bacilli in non-pulmonary tuberculosis. Brandt(51), in Norway, examined 263 specimens from bone and joint lesions and only recovered two bovine strains, while from twenty-two lymph glands he only recovered one. Groh & Renaté(52), in Germany, examined fifty-three strains from urinary tract infections and found that 42·4% were due to organisms of the bovine type. In the same country, von Vararhelgi(53) examined the fluids from forty-eight cases of tuberculous meningitis and recovered six, or 12·5%, of bovine strains.

Several advances have been made in the bacteriological technique for the diagnosis of tuberculous infections, which might be applied to specimens from both human and bovine sources. Pryce(54) has devised a new film-culture method for the early observation of growth. A film of sputum is dried on a glass surface, treated with 5–25% sulphuric acid, washed with water, and incubated with haemolysed blood. The technique may be carried out on the floor of Petri dishes, in vaselined rings on microscope slides, and in other ways. After 7 days' incubation the preparation is washed, stained by the Ziehl-Neilsen method, and examined with the lower power of the microscope. The method seems to be applicable to various types of specimens, but its value as a diagnostic procedure has not been established, as comparative tests with other methods have not been presented.

Fluorescent microscopy has also been employed to help in the recognition of tubercle bacilli in specimens. Excellent reviews of the application of fluorescent microscopy to biology have been made by Ellinger(55), and the application of near-ultra-violet rays and fluorescence phenomena as aids to discovery and diagnosis by Figge(56), while the basic technique of colour photography of fluorescence phenomena has been studied by Figge & Clark(57). The technique of the method is as follows: a source of ultra-violet light is necessary, and a blue ultra-violet transmitting filter placed in front of the light source is essential. The light-transmitting quality of the filter has not been defined in terms of wave-length. A monocular microscope must be used, with an 8 mm. objective and 20 × ocular or 1·8 mm. objective and a 6 × ocular. An aluminium-surfaced mirror is placed in front of the ordinary microscope mirror. Finally, a light-yellow filter, complementary to the blue-light filter, is placed in the ocular of the microscope. Again the filter has not been defined in terms of transmission of light. Smears are made in the usual way, and are then stained in a solution of auramine (0·1 g. of auramine, 3 c.c. of liquefied phenol, and 97 c.c. of distilled water) for 2 min., washed and decolorized with acid alcohol for 2–4 min., and then redecolorized in a fresh solution of acid alcohol, washed and dried, and are then ready for examination. The acid-fast bacilli appear as small, bright-yellow bacilli against a dark background. Satisfactory results obtained by the use of this method in the examination of sputum and other

specimens have been reported by many workers, the main advantage being the saving of time in the examination of each smear. The provision of suitable apparatus and much more extensive controlled observations are necessary before a final opinion can be expressed on its true value.

Several papers have dealt with the relative virulence of human and bovine types of tubercle bacilli in animals and man. Griffith(58) states that the three important points in determining the virulence in animals are dosage, vitality of the culture, and experience of the observer. He believes that the bovine type of bacillus is at least as virulent for man as the human variety. He has cultivated bovine bacilli of attenuated virulence from human lesions of bones, glands and peritoneum, but the most prolific source has been lupus vulgaris, and in 204 such cases 102 were due to the bovine variety and only forty-one of these were fully virulent. In general, the longer the disease has persisted, the more likely are the bacilli to be reduced in virulence for test animals. In several cases the diminution of virulence has been observed in successive examinations, and the maintenance of virulence during years of cultivation on media varies with different strains. Again, Munro(59) isolated the bovine type of *Mycobacterium tuberculosis* from human sputa in fifty-eight cases out of 1160. An analysis of his data fails to support the theory that the bovine-type of tubercle bacillus is less virulent for man than the human type, since the death-rate was twenty-two out of thirty-eight in the females and twelve out of twenty in the males. It is suggested that in bovine-type infections the duration of a fatal illness is usually short. If, however, infection is arrested, the disease may persist over a long period, with attenuation in virulence of the organism.

The vole and hamster have been studied further as experimental animals for tuberculous infections. Thus, Griffith(60) found that when the vole was used in an attempt to differentiate bovine and human types of tubercle bacilli, bovine strains caused a rapidly fatal progressive disease, accompanied by great multiplication of bacilli, whereas human strains rarely provoked visible lesions. It is not possible to distinguish between dysgonic and eugonic human types. Hamsters were tested by subcutaneous inoculation and by the feeding of bovine, human, avian and vole types of tubercle bacilli; the human strains produced generalized lesions when injected, but only slight changes or no lesions after feeding; the bovine strains, on the other hand, produced generalized disease after feeding; avian were less virulent though they multiplied profusely in the organs. The vole bacillus gave rise to lesions which were characterized as epitheloid in character, without caseation or necrosis.

Brooke(61) described the cultural characteristics and pathogenicity to small animals of the vole strain. Serologically this strain could not be differentiated from human and bovine strains, but could be differentiated from avian and other acid-fast organisms.

A number of papers have discussed immunity to tuberculosis. Thus, Gloyne(45) notes that in planning experiments on immunity to tuberculosis, too little attention has been devoted to the question of the route and amount of infection and the effects of frequent exposures. He believes that, as far as possible, natural routes should be followed in applying test doses. The subject has also been fully covered in an editorial in the *British Medical Journal*(62), and by Kayne(63). The latter author notes that the same evidence was brought forward in 1925 as to-day on the seriousness of the problem of control of milk-borne tuberculosis, and states that it is most disturbing to conclude that apparently little, if anything, had been done about it at all since then. Finally, he condemns the view that partial immunization against pulmonary tuberculosis is attained by the ingestion of small numbers of bovine tubercle bacilli in milk, and hopes that such a travesty of one aspect of immunity will not be allowed to hinder an effective campaign for the eradication of tuberculosis in cattle.

Typhoid and paratyphoid fevers

A detailed account of the incidence, diagnosis, methods of detection of source and spread of infection, and the part played in their dissemination by water, milk and food has been given by Scott(64). Furthermore, Felix(65) has emphasized the value of typing of typhoid bacilli by means of Vi bacteriophage for the purpose of epidemiological investigation. The distribution of the various types found by Felix are as follows:

Showing the Vi-phage types of Bact. typhosum *found in England, Wales and Scotland during* 1940–2

Craigie's original Vi-phage types	No. of patients and carriers from whom typhoid strains belonging to the various Vi-phage types have been isolated					
	England and Wales			Scotland		
	1940	1941	1942	1941	1942	Total
	Part 1					
A	15	18	50	3	7	93
B2	—	—	5	—	—	5
C	2	2	39	—	1	44
D1	1	4	10	1	—	16
D2	1	16	—	—	—	17
E1	17	19	57	—	16	109
F1	4	3	9	—	—	16
F2	2	1	—	2	7	12
J	—	2	—	—	—	2
'Imperfect' Vi forms	30	30	57	1	—	118
'O' form (Vi-negative variant)	1	—	6	—	1	8
Total	73	95	233	7	32	440
	Part 2					
	Some of the 'Imperfect' Vi forms recorded in Part 1 were successfully typed by means of four new Vi-type phages					
New Vi-phage types						
D4	—	11	8	—	—	19
D5	—	—	1	—	—	1
L2	5	—	—	1	—	6
No. 91	2	10	11	—	—	23
Total	7	21	20	1	—	49

As Felix states, without this new laboratory aid there can be little hope of determining the source of infection of many of the sporadic and small groups of cases that occur throughout the country. This fact is well illustrated in a paper by Bradley(66), who was able eventually to trace the source of infection in sporadic cases to a carrier living 100 miles away from most of the cases, all strains of typhoid isolated being type D4. The primary cases were distributed as follows:

No. of cases	Year	Month	Location
1	1941	January	Herts
8	1941	February	Bucks
2	1941	March	London, Bucks
3	1941	April	London, Bucks
1	1942	April	Kent
1	1942	June	Wilts
5	1942	June	Bucks

The investigation suggested that milk was the vehicle of infection, but, although the 1941 cases were supplied by six different retailers, all obtained the milk from a depot in Herts which had four sources of supply, one of them being a group of thirteen farms in Wilts. With the occurrence of typhoid fever in a Wilts farm labourer in 1942, the milk from a certain farm became suspect, and at this dairy farm the farmer himself was found to be a chronic carrier of type D4 typhoid bacilli.

Besides the possibility of direct contamination by a carrier, milk may be infected through the water. Meyer *et al.*(67) reported an outbreak in St Louis, U.S.A., due to this cause, where the public supply was infected by back siphonage from a sewer. Sirois(68) describes an outbreak involving sixty-eight individuals, with seven deaths, or a mortality rate of 10·3%, and 45% of the cases occurred in children under 10 years of age. The dairy produced 70 qt. of milk a day and supplied 240 persons in all, and 27·5% of these suffered from the disease. The dairy farmer's

family had been ill with typhoid and infected the supply. Watt(69) reported on a small outbreak of twenty-six cases, with four deaths, near Auckland in New Zealand, where the supply had been infected by a carrier on the farm. The *Journal of the American Medical Association*(70) contained a report on an outbreak of typhoid fever in a suburb of Melbourne, Australia, in March 1943. The epidemic was a very large one involving 440 individuals and had a mortality rate of 5%. The outbreak was traced to milk which was distributed through one particular dairy which provided milk from various farms, and the source of infection was traced ultimately to a female typhoid carrier. Needless to say the milk was not pasteurized. The incidence of the disease was highest in the age group 5–14 years. Infants under a year were apparently immune, and another interesting feature was that no returned soldier from the war of 1914–18 developed the disease, although over 200 were exposed to the risk of infection.

Milk products may disseminate typhoid organisms, and this is well illustrated in two outbreaks. The first is described by Gauthier & Foley(71), and in it forty individuals were involved. The usual foodstuffs were excluded as the cause of the outbreak, but each of the patients gave a history of having eaten Cheddar cheese manufactured in one particular factory from raw milk. Conclusive proof was obtained as the result of one doctor in Montreal buying 15 lb. of this cheese, and no less than seven individuals who ate it developed typhoid fever. Further investigation showed that eighty farms supplied milk to the factory, and at one farm a known carrier had assisted in the milking. This woman was a carrier of D1 phage-type typhoid bacilli, and all patients in the outbreak were infected with the same type. It was concluded, therefore, that this carrier was the cause of the outbreak. The second epidemic is described in the *American Journal of Public Health*(72), and occurred in California. The investigation into this outbreak elicited the information that the common food factor was unripened cheese of the Romano Dolce type. It was found that over 90% of the cases had taken this type of cheese. An act was immediately passed by the State Legislature making it compulsory for all milk which is to be used in the preparation of cheese to be pasteurized. Attempts to define the limit of viability of *B. typhosus* in Cheddar cheese have been described by Campbell & Gibbard(73). The different strains belonging to phage types C.F. and M. were used for the experiments. The organisms were added to the milk, and cheese was prepared by the usual method in an experimental vat; the cheeses were ripened at 58–60° F. for 14 days and thereafter sampling was commenced; the counts of *B. typhosus* in the milk before the preparation of the cheeses ranged from 1000 to 600,000 per c.c., but during the ripening process large numbers of bacteria had died off. By using as much as 5 g. of cheese and tetrathionate broth as the enrichment medium, typhoid bacilli could be demonstrated in the cheeses after being stored at 40–42° F. for 336 days and, after storing at 58–60° F., up to 196 days. The experiments were concluded through lack of material, but the organisms could have survived for longer periods.

Felix & Callow(74) have developed the phage typing of *B. paratyphosus* B in a manner similar to that employed for *B. typhosus*. Antio-O bacteriophages have been found to act on various members of the *Salmonella* group, but anti-Vi bacteriophages can be adapted to develop a high degree of specificity for particular strains. Four separate and distinct Vi-phage types and subtypes 1, 2, 3*a* and 3*b* have so far been identified, and the procedure will assist greatly in epidemiological investigations.

In 1940 there occurred at Kettering an outbreak described by Hogg & Knox(75) of 222 cases of paratyphoid fever, the source being cream cakes from one particular bakery. 52% of the cases had consumed cream confectionery, 66% cream confectionery or cakes, and 70% cream confectionery, cakes, or bread. Two previous epidemics of paratyphoid fever had occurred in the town and surrounding districts in 1927 and 1936, and on both occasions there was evidence pointing to the bakery as the source of infection. In the 1940 epidemic, specimens were obtained from thirty-two male employees, and no less than ten of them were found to show evidence of infection, though only five had had clinical symptoms of the disease. Parry(76) reported a small epidemic of some seventeen cases in a rural district of Worcestershire. Most of the cases arose as the result of a milk supply being contaminated with sewage effluent through the water supply used for

dairy purposes. The infecting organism was recovered from this water supply but not from the milk. Savage(77) has studied paratyphoid fever from the point of view of its epidemiology, and finds certain differences from the epidemiology of typhoid fever. The incubation period is more variable and the mortality rate is very low, while the symptoms in some cases resemble a *Salmonella* infection, and the seasonal incidence is definitely a summer prevalence. Cream takes pride of place as a vehicle of infection, although other types of foods are often incriminated. The disease is rarely spread by water or shellfish, and the vehicle infectivity rate is often very low. Savage is inclined to the belief that the importance of the chronic carrier in this disease had been overstressed, and in his experience it is the transitory carrier who is the chief source of infection.

Salmonella food-poisoning infections

This group of bacteria becomes more numerous and complex. Many new members have been added, and epidemiological investigations have shown their widespread distribution and the multiplicity of lower animals and birds which are affected by them. Henning(78) and Henning & Haigh(79) have published a very extensive paper on the antigenic structure of the Salmonellas obtained from domestic animals and birds, and Bornstein(80) in the United States has published an extensive review of the whole problem of Salmonellosis, while, in the same country, Edwards & Bruner(81) have given the results of their investigation into the occurrences and distribution of *Salmonella* types. The serological recognition of members of the *Salmonella* group has been well described by Bridges & Taylor(82). This paper gives details of the methods to be adopted, and describes the constitution of the various typing sera available at the Standards Laboratory, Oxford.

Four outbreaks of *Salmonella* infections due to milk have been described. Merkel(83) has published a paper on an outbreak due to milk which affected thirty individuals with two deaths in children. The outbreak occurred at an asylum at Sudbaden, and the symptoms were those of an acute enteritis. Sixty-eight strains were isolated and were found to be *S. hvittingfoss*, thirty-one being in the A phase and fifteen in the B phase. Sutherland & Berger(84) have published an account of a large milk-borne outbreak involving at least 162 persons. This outbreak occurred in the Knaresborough District of Yorkshire, and was due to milk infected with *Salmonella* type Dublin. The incubation period of the infection was 14–20 hr., and the symptoms which followed were those of acute gastro-enteritis. Six people were resident at the farm and three of those also showed similar symptoms. Two labourers remained well, but their families were affected. The dairy herd consisted of twenty cows and there had been no recent additions to the stock. The milk from the farm contained *S. Dublin*, but milk samples taken from each individual cow failed to show the presence of the organism. Whey agglutination tests against *S. Dublin* showed a positive result in one instance in a titre of 1/32, and subsequent examinations of the dung from this cow showed the presence of numerous colonies of *S. Dublin*.

Dysentery

During recent years there has been a marked increase in the incidence of diarrhoeal diseases, and this is rather in contrast to what has happened in connexion with other types of infectious diseases. In England and Wales there has been a definite upward trend in the deaths from this cause, from 1812 in 1940 to 2569 in 1943. Further, notifications of actual cases of dysentery increased from 2843 in 1940 to 7772 in 1943. In Scotland, Sutherland(85) has reviewed the incidence of the disease since 1931. He found that between the years 1931 and 1935 the incidence was of low prevalence, and that during the years 1936–41 there was a very high prevalence, but the deaths from dysentery have not, in general, paralleled notification increases. The late summer has been the time of highest incidence. Most of this disease has been largely due to contact infections, but some epidemics due to milk have been recorded in this country and abroad.

Steuer(86) has investigated the viability of dysentery bacilli in milk. In sterilized milk, Shiga and

Flexner dysentery bacilli have been found to survive for 20 or more days at 37° C., room temperature, and in the ice chest. In raw milk the period of survival is less, varying from 1 to 15 days according to the temperature at which the milk is held. If at 37° C., the dysentery bacilli may not survive 24 hr., whereas, if the milk is held at −3° C., they continue to exist for a very much longer period. The factors concerned are temperature, acidity, and the bacterial flora of the milk.

Faulds(87) described an outbreak of dysentery in which one dairy farm supplied 90 pt. to seventy-two customers, of whom forty-three developed symptoms of severe diarrhoea and vomiting. From many of the cases *Bact. Sonnei* was isolated. The farm was occupied by a grandfather, his son, his son's wife, and his granddaughter. The grandfather and his daughter-in-law had severe diarrhoea, and the infection was proved to be due to *Bact. Sonnei* also. The causative organism was not demonstrated in the milk itself, but this is not surprising since contamination from human cases and carriers is bound to be sporadic. The milk supply could not be stopped, but customers were advised to boil the milk, and the outbreak ceased. The same author(88) described a further outbreak of dysentery in which 120 cases were involved, and all obtained their milk from one retail dairy. The organism isolated from the patients was again *Bact. Sonnei*. Specimens were obtained from all workers at the retail dairy and from the workers at the two farms which supplied the dairy. At one farm which produced T.T. milk, children had suffered from diarrhoea, and the same organism was isolated from one child and from several workers. At the dairy two workers gave a history of having an attack of enteritis, and from both *Bact. Sonnei* was obtained. The customers were warned to boil the milk until such time as arrangements could be made for its pasteurization. Thereafter the outbreak quickly subsided.

Undulant fever

The various papers which have been published during the past few years indicate that undulant fever is becoming more world-wide in its distribution. Cases have been reported for the first time from some countries, while in other areas there appears to be an increased prevalence.

In Great Britain several papers record the occurrence of small outbreaks and isolated cases. Elkington *et al.*(89) described an outbreak in a school attended by 400 boys, where each pupil received daily a glass of ungraded milk. Within a period of 3–7 weeks there occurred two definite clinical cases of undulant fever, and in addition twenty-six boys suffered from a transient illness suggestive of mild undulant fever. In ten instances amongst these twenty-six boys, their sera gave a positive agglutination test in dilutions ranging from 1/20 to 1/1000, and the authors estimate that 30% of the boys suffered from latent infections. The outbreak ceased when the raw milk was replaced by pasteurized. Again, Cruickshank & Stevenson(90) described the occurrence of four cases of undulant fever at a school attended by fifty-four girls between the ages of 9 and 17, with a staff of fourteen teachers and maids. The milk supply for the school was obtained from a farm in which contagious abortion had occurred.

A small outbreak(91) of four cases of undulant fever occurred in a girls' school at North Molton. Their ages varied from 9 to 16 years. The milk supply was obtained from a pedigree Guernsey herd in which there had been contagious abortion a month or so previous to the human infections. After this occurrence, the milk was no longer consumed in the raw state but was boiled, and thereafter there were no further cases. Leys(92) described eight sporadic cases of undulant fever occurring in the northern area of Scotland. The disease was on the whole benign, but gave rise to considerable difficulty from the point of view of differential diagnosis. In Germany, Niedenführ(93) estimated that the number of people who contracted undulant fever annually in Germany varied between 626 in 1930, 146 in 1935, and 416 in 1939. In 1939 the proportion of male cases to female ones was 313 to 103, and the age group with the largest number of infections was 30–39 years.

A number of accounts of the incidence of the disease come from the United States. Feemster & Hyder(94) give an account of the occurrence of undulant fever in Massachusetts. During the period 1935–40, the annual number of cases ranged from forty-two to fifty-five, and there was no marked seasonal prevalence. The sex ratio of the cases was five males to two females, and the

disease was more prevalent in areas where there was no pasteurization. 82% of the patients used raw milk. Westerman(95) gives an account of undulant fever in Kansas, where the first case occurred in 1929, but in 1940 the number had risen to eighty-two. Most of the cases were in the age group 30–50 years, but the youngest patient was only 4 years. The author concludes that the majority of cases acquired their infection from drinking raw milk. Terry(96) discussed brucellosis as a public health problem in Texas, and mentioned that it is now more than 35 years since Craig described the first authentic case in the U.S.A. In 1925 only twenty-four cases were reported, but in 1940 the number notified was 3368. This probably means that there was no marked increase in actual infections, but the disease was now more efficiently diagnosed. In the mid-west and south-west States there has been a continued high incidence. In Texas, for instance, forty-three cases were recognized in 1933 and 354 in 1940. Guilford(97) records that in Wisconsin, out of a total of 894 persons who had suffered from undulant fever, 813 had consumed raw milk: of these, 436 had contact with farm animals, and probably 33% had obtained their infection from milk. Only seventy-five cases occurred in persons drinking pasteurized milk, and of these, forty-nine had contact with animals, mostly in abattoirs. In the remaining twenty-six the source of the infection was obscure. The disease was practically confined to adults, and the sex ratio was 2·4 males to 1 female. Borts *et al.*(98) reported an unusually large epidemic of brucellosis involving seventy-seven persons in the State of Iowa. The organism was recovered from the blood of thirteen patients out of twenty-nine, and the strain was identified as *Brucella suis*, and this was confirmed by Huddleson. The cause of the outbreak was an infected dairy herd in which there were forty-three cows. Four of these animals gave positive agglutination tests, and from three *Brucella suis* was recovered from the milk. On the farm, cows and pigs were allowed to mix freely, and fourteen out of twenty-four sows also gave a positive agglutination test. Jordan *et al.*(99) record that during the years 1930–41, 29,594 cases of infection were notified in the forty-eight States of the U.S.A., giving an incidence rate of 1·87 per annum per 100,000 of the population.

In Latin America increasing attention is being paid to the clinical and epidemiological aspects of brucellosis. In Mexico, Castaneda(100) records that 95·2% of human cases are due to the *melitensis* variety, whereas 3·5 and 1·3% were caused by *abortus* and *suis* respectively. The infection is apparently very high in goats, and 25–30% of cattle and about 35% of swine are affected. The author estimated that at least 3% of the human population suffers from the disease. Another report from Mexico by Villalobos(101) states that in the district of Chihuahua there is evidence of increasing infection of goats and cows, and as a result there has been a corresponding increase in human cases from twenty-four in 1939 to forty-five in 1942. Measures for the control of cheese made from goats' milk have been made operative, and further proposals include elimination of reactors and vaccination of stock.

Reports from South America by Goobar & Illia(102) and Stuckert(103) suggest a considerable increased prevalence of the disease. Both papers deal with the situation in the Cordoba Province of Argentina. In 1941 it was thought possible that the incidence in man was as much as 40%. Examination of sera from goats showed a particularly high incidence of infections, and the disease was virulent for cattle as well. The human cases had often a severe infection, with a fairly high fatality rate.

Again, in Uruguay, Purriel, Risso & Espasandin(104) have published a monograph in which they state that the *abortus* variety is quite prevalent but that the other varieties are not. The disease in human beings is, in nearly all cases, of the occupational type, the incidence varying in direct relation to the association of men with cattle slaughtered. Milk-producing cows show a 50% rate of infection.

In such widely separated countries as the Tchad region of French Equatorial Africa and Vizagaputan in India, Moustardier(105) and Ceccaldi & Guilhamon(106) and Pardali & Raman(107) report the recognition of the first cases of undulant fever due to the *melitensis* variety.

Investigations into the incidence of *Br. abortus* in milk in Great Britain continue to be made. Thus Duke(108), in the course of an investigation into the incidence of *Br. abortus* in milk, isolated

one strain from an accredited herd of seventy-six cows, which conformed to the *melitensis* type. For the previous 14 years no goats had been kept on the farm. The organism, which was obtained after inoculation from guinea-pig tissues, grew well on liver agar in primary cultures, producing no H_2S, while it also grew abundantly on media containing the dyes thionin, fuchsin, methyl violet and pyronin. The strain was agglutinated by a *melitensis* serum but not by a non-specific *abortus* one. From single samples of milk from the cows in the herd, three *Br. melitensis* strains were isolated. Menton(109) has examined over 1000 milk samples from the midlands of England, and of these, 34·5% showed whey agglutinins. When, however, these samples were inoculated into guinea-pigs, only 29·1% showed *Br. abortus*, but, on the other hand, 9·1% of samples which showed no agglutins contained living *Br. abortus*. The sera of the guinea-pigs inoculated with the milk samples were examined at 3-, 6- and 9-weekly periods after inoculation, and this method was found to give a higher percentage of positive results than if only one examination had been made at 6 weeks. Again, Jones(110) examined 314 samples of milk obtained in the Liverpool area during 1933–4 and 408 samples from Kent in the years 1939–40. In the Liverpool series *Br. abortus* was found in forty-eight samples, while in Kent fifty-nine samples gave positive results. There was some indication of a higher incidence of *Br. abortus* in milk in January and a low incidence in August. On the other hand, when samples were obtained from individual cows or small groups of cows, this seasonal variation was not in evidence.

In the U.S.A, in the course of the examination of 1788 milk samples, Bradley *et al.*(111) encountered 262 which gave a positive whey agglutination test. When these positive samples were cultured on Bacto-tryptose agar and on liver infusion agar, twenty yielded a positive result. In plate isolation, crystal violet was added to Bacto-tryptose agar in a concentration of 1 in 200,000. This concentration of dye did not inhibit *Br. abortus* but produced practically complete inhibition of Gram-positive contaminants.

In Canada, Fulton(112) has found that *Br. abortus* could remain alive in sterile milk for 18 months in the ice-box, and for 10 months at room temperature in the dark. When *Streptococcus lactis* was added to milk as well as *Brucella abortus*, *Br. abortus* did not survive more than 5 days. In unsalted butter *Br. abortus* survived for 13 months, and when 2·1 and 1·17% salt were added, for 6 and 7 months respectively.

Comprehensive monographs have been published by Huddleson(113) and Harris(114) in the U.S.A., and the reader is referred to these for full details of clinical, bacteriological, and epidemiological findings. Both books also contain extensive bibliographies.

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